GENETIC PREDICTION OF FEMALE REPRODUCTIVE PERFORMANCE FROM MALE CHARACTERISTICS.

By

ABUL BASAR MOHAMMED MAFIZUL ISLAM

M.Sc.(A.H.), (Bangladesh Agril. Univ.)

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Institute of Animal Genetics,
Edinburgh.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Reproductive rate has always been recognised as a major item in any livestock enterprise. The main objective of improvement in reproductive rate is to increase the number of offspring produced from individual animals and thereby to improve efficiency in production in terms of animal products. The level of reproductive rate has an important bearing in ensuring stock replacement, producing surplus stock for marketing and obtaining a higher selection differential in any selection programme for breeding stock.

The reproductive performance of males in mammals is dependent upon age of puberty, libido, quality of semen production, fertilizing capacity of sperm produced and length of reproductive life. The reproductive efficiency of females is determined by age of puberty and conception, frequency of parturition, number of young born per parturition, mothering ability in rearing the young produced and the duration of reproductive life. Among them, frequency of parturition and the number of young born per parturition, termed litter size, have a large effect in the reproductive rate and hence on efficiency of production. Improvement in litter size of individual females in polytocous animals may lead to overall improvement in reproductive efficiency. The relative productive and economic values of litter size in sheep have been emphasised by many investigators (Blaxter, 1968; Purser, 1969; Dickerson, 1970; Large, 1970) and it is recognised that litter size is one of the most important factors responsible for efficiency of production in sheep.

Problems in genetic improvement of reproductive performance

Studies in the genetic improvement of litter size in sheep indicate that it can be changed by selection, but the response to selection is very slow. Wallace (1964) obtained a response of 0.017 lambs per ewe per year between selected and unselected control line in Romney sheep. Turner (1969) reported an annual divergence of 0.023 lambs born per ewe between the lines selected for high and low litter size in Merino sheep. Selection for litter size in the mouse showed an average divergence of 0.16 young per generation between the high and the low lines selected for 20 generations with intra-litter selection (Falconer, 1960,a) and a divergence of 0.15 young per generation between the selected and the control lines with intra-litter selection for 10 generations (Bradford, 1968).

Two main factors are responsible for this slow response in two different species. Firstly, the heritability of the number of young born per parturition is low, around 10% in the sheep (Turner, 1969; Bradford, 1972) and probably 20% in the mouse (Falconer, 1964; Bradford, 1968) and secondly, the trait is sex-limited. In a situation where the trait is sex-limited, the genetic gain in selection is reduced to nearly half because selection can only be applied in one sex. The situation becomes worse in the case of sheep because litter size cannot be measured until two to three years of age. The low reproductive rate itself limits the selection pressure which can be applied and selection in most populations is essentially a choice between males which cannot be measured directly. The possibilities of improving the rate of response are therefore limited. According to the theory of quantitative genetics, three points can be considered for increasing

the rate of genetic improvement in reproductive traits, firstly by increasing the selection intensity by overcoming the sex-limitation, i.e. to select individuals simultaneously in both sexes, secondly, by increasing the accuracy in selection which is the correlation between the index on which the animal is selected and the breeding value of the trait which is to be improved, thirdly, by reducing the generation interval by measuring individuals at early ages.

Selection of males on the basis of reproductive performance of their female relatives such as mother's or sisters' performance has low accuracy in predicting individuals' genetic merit. Progeny testing for reproductive performance is a laborious and lengthy process and provides a long generation interval. The use of indirect selection on arbitrary quantitative or qualitative traits such as body weight, face cover, skin fold, blood group, etc. have very limited value in improving reproductive performance, probably because of low genetic relationships and lack of direct physiological relationships with the reproductive traits (Turner, 1969). Turner discussed the possibility of indirect selection for reproductive performance in sheep and concluded that it might be better than nothing when there is no reproductive record.

Alternative possibilities for genetic improvement in reproductive performance

One possible method for increasing genetic improvement in reproductive performance is to select for physiological variables which influence the reproductive performance. The number of young born is determined by the number of eggs available for fertilization and the losses between fertilization and parturition. Selection for litter

size led to a positive correlated change in ovulation rate (Packham and Triffit, 1966; Bindon et al., 1971; Trouson and Moore, 1972), indicating an association between ovulation rate and litter size in sheep. Superovulation (Newton et al., 1970) and embryo transfer in excess of normal ovulation rate (Moore, 1968; Bradford et al., 1971) invariably leads to increased litter size in sheep which provide evidence that ovulation rate is the primary factor limiting the litter size in sheep. The ability of the females in sheep to produce a considerably higher average litter size when the number of eggs or embryos are increased apparently differs from the situation in pigs (Bazer et al. 1969) and in mice (Land and Falconer, 1969; Bradford, 1969) where natural ovulation rate on average provides females with as many embryos as they can carry. It seems that the sheep has apparently evolved an ovulation rate which frequently provides less than the maximum load they are capable of carrying under good health and nutrition. At the present level of performance, litter size appears to be limited by the number of fertilized eggs available for implantation and increases in the ovulation rate resulting in increased litter size in sheep. The relationship between different aspects of reductive activity is in general positive. The frequency of ovulation is found to be correlated with mean ovulation rate and the duration of oestrus is correlated with ovulation rate and litter size (Land, 1970,a; Land et al., 1973). The number of eggs shed and the regularity of its incidence are dependent upon the activity of the ovary and it is assumed that increases in ovarian activity will lead to simultaneous changes in several components of fertility and a general improvement in performance.

The activity of the ovaries is controlled by the gonadotrophic

hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by the anterior pituitary gland. The number of developing follicles in the ovary, production of steroid hormones, display of oestrus and the number of eggs shed are all dependent upon the activity of FSH and LH and the response of the ovary to the stimulus of these hormones. Animals with high levels of both should have high litter size and better reproductive performance. Measurements on the concentration of hormone in the pituitary gland as well as in the urine at different stages of the oestrus cycle in sheep indicated that the secretion of luteinizing hormone was related to the ovarian activity of the breed type (Land, Crighton and Lamming, 1972). The same effect measured in young female lambs indicated that the duration of interval between injections and release of LH was longer in breeds which have a higher ovulation rate (Thimonier, Pelletier and Land, 1972). There is evidence that the plasma LH activity of young Merino rams is related to the ovulation rate characteristic of their strain type (Bindon, 1973). It may, therefore, be possible to base genetic selection for improving the reproductive performance in sheep on some aspects of gonadotrophic activity.

Probable genetic relationship between the male and the female fertility

A relationship between the fertility of males and that of females is possible in view of the common physiological pathways underlying the expression of male and female reproductive activities. It is well known that the same gonadotrophic hormones, FSH and LH, are involved in the physiological control of reproductive activities in both sexes (Cole, 1969). In the male, these control the growth and development of testes,

rate of spermatogenesis and production of testosterone which influences the development of secondary sexual characteristics and male libido. In the female, these control the growth and maturation of the ovaries, the development of follicles and production of ova, production of steroid hormones, display of oestrus, maintenance of the sexual cycle and pregnancy. Gonadotrophic hormones therefore play a central role in the contribution of both males and females to successful reproduction, influencing the ovarian activity in females and testicular function in males. Furthermore, with the exception of one sex-chromosome, the inheritance of an individual is independent of its sex, so that all autosomal genes controlling the gonadotrophic production and its activity are common to both sexes. So, the quantitative expression of the male and the female reproductive activities should be genetically correlated. The transmission of genes for female fertility through the males is demonstrated by differences in reproductive performance of the daughters of different sires in sheep (Purser, 1965) and in mice (Land and Falconer, 1969). The variation in the date of first oestrus in ewe lambs between the progeny of different sire groups also indicate the effect of the sire in transmitting genes for the activity of the ovaries of unmated non-lactating females (Purser, 1972).

It might therefore be possible to predict the reproductive performance of female sibs and of a male's potential daughters from the measurements of reproductive characteristics of males. Such a common variable may be gonadotrophic hormone level or the activity of target organs such as testis growth and size, rate of spermatogenesis or male libido. The quantitative measurements of the reproductive characteristics in males may be incorporated into a selection programme for

genetic improvement in female reproductive performance.

The main advantage of such endocrinological parameters is that these can be measured in relatively young individuals and selection can be applied simultaneously in both sexes, e.g. selection for testis activity in the male and selection for ovarian activity in the female. Selection in both sexes and in young individuals would have a marked effect on the selection intensity and generation interval, particularly in a species such as the sheep where the reproductive rate is low.

Based on the evidence described above, the present study was designed to investigate the phenotypic and genetic properties of testis growth, size and activity in the mouse and in the sheep. The main object of the study was to evaluate the genetic effects of testis size on the components of female fertility in the mouse as a theoretical base and to elucidate the possibility of its use as a genetic predictor of female reproductive performance in the sheep. In the mouse, a two-way upward and downward selection for testis size was carried out and the correlated responses to selection in components of female fertility were studied (Chapter 2). In the sheep, seasonal variation in testis size and rate of sperm output was examined in different breeds (Chapter 3).

CHAPTER 2

SELECTION FOR TESTIS SIZE IN MICE

I. INTRODUCTION

The quantitative physiological relationships between reproductive activity in males and females which are considered to be mediated through the level and activity of gonadotrophic hormones may be measured in the end products of gonadotrophic stimulation. Such traits are growth of testis, rate of spermatogenesis or level of libido in males and ovulation rate, oestrus duration or incidence of oestrus in females. Testes growth is mainly dependent on the stimulus of gonadotrophic hormones. The growth of testes has been used as a bio-assay for FSH (Loraine, 1958). Testes do not grow in hypophysectomized immature males whereas with replacement therapy with FSH and LH shows that they grow relatively normally (Woods and Simpson, 1961; Lostroh, 1969). Studies on the growth rate of testis in hemi-castrated males (Duff, Dame and Renfro, 1968; Courot, and Carr, 1971; Land / 1975) showed an accelarated growth of single testis relative to the control groups. The increased growth rate of testis observed in the hemi-castrated males might be due to the stimulus of a similar amount of gonadotrophin acting on a single testis. The evidence indicates that testes growth and size in males may be used as a simple measure of circulating gonadotrophins, which in turn should be an indication of the ovarian activity of their female relatives.

It was reported that the testis weight of contemporary male mice changed in the same direction as the ovulation rate in females following twelve generations of direct selection for natural and induced ovulation rate (Land, 1973). The divergence between lines selected for high and low ovulation rate was statistically significant. The change in testis

weight following selection for ovulation rate indicated that the traits were genetically correlated and had a common physiological component. The correlation between mean testis weight and mean ovulation rate of the selected lines was shown to be 0.97 and the between lines regression of testis weight on ovulation rate was 9 mg. per egg. The partial correlation between testis weight and ovulation rate for constant body weight was estimated to be 0.82, indicating that the removal of variation in body weight had a small effect on the magnitude of the relationship.

These findings indicate that testis size could be used as a selection criterion in males for genetic improvement of reproductive performance in females. There is also evidence that a major portion of variation in testis size is genetic. Investigation on strain differences in testes weight in mice (Shire and Barte, 1972) indicated that the differences between strains were relatively unaffected by environmental variation. Measurements on hybrid mice confirmed that much of the observed differences in testis size was genetic in origin.

Following the evidences, the present study is designed to investigate the genetic nature of testis size and to elucidate the effects of selection for testis size in the mouse. It is postulated that selection for testis size would apply selection pressure to common intrinsic factors responsible for reproductive performance in both sexes and hence would demonstrate the transmission of genes for female fertility by males through common physiological pathways of reproductive activities.

The main objective of the study is to evaluate the direct changes brought about by selection on testis size and its effects in changing the components of female fertility as correlated responses. The correlated changes in body weight following selection for testis weight are

also under consideration. Two components of female fertility are considered, one is the ovulation rate which should include the factors for ovarian activity and the other is litter size at birth, which may demonstrate the fertilizing capacity of the male and uterine capacity and embryo survival in the female. Litter size at weaning is also under consideration as it indicates the mothering ability of the female.

II. MATERIALS AND METHODS

(i) Experimental Mice

The experiment was carried out in the mouse house of the A.R.C. Unit of Animal Genetics, Edinburgh. The stock of mice used in the experiment was taken from the Control lines of the replicated selection experiment for body weight in the Q strain (Falconer, 1973), and all individuals came from the 39th generation of A, B, E and F Control lines. At the beginning of the experiment 30 males and 30 females were randomly taken from each Control line out of 10 to 14 full sib families. Individuals of A and B Control lines were similar in age and so were the individuals of E and F Control lines. EF groups of mice were approximately two weeks younger than AB groups of mice. Standard practices of management such as feeding, rearing and maintaining of mice were followed throughout the experiment.

(ii) Selection Programme

The programme of selection was based on individuals' testes weight taken in milligrams. A two-way upward and downward selection was undertaken. No Control group was maintained. Divergent selection was started in two replicates, one from the reciprocal crosses of A and B (AB), and the other from E and F Control lines (EF). The selected lines

were designated as ABH and ABL in one, and EFH and EFL in the other, representing high and low testis weight, respectively in each replicate. Random mating was practised in each generation avoiding full sibs mating. Matings consisted of one male and one female individuals. Approximately one-fourth male individuals selected from total number of males recorded in each generation.

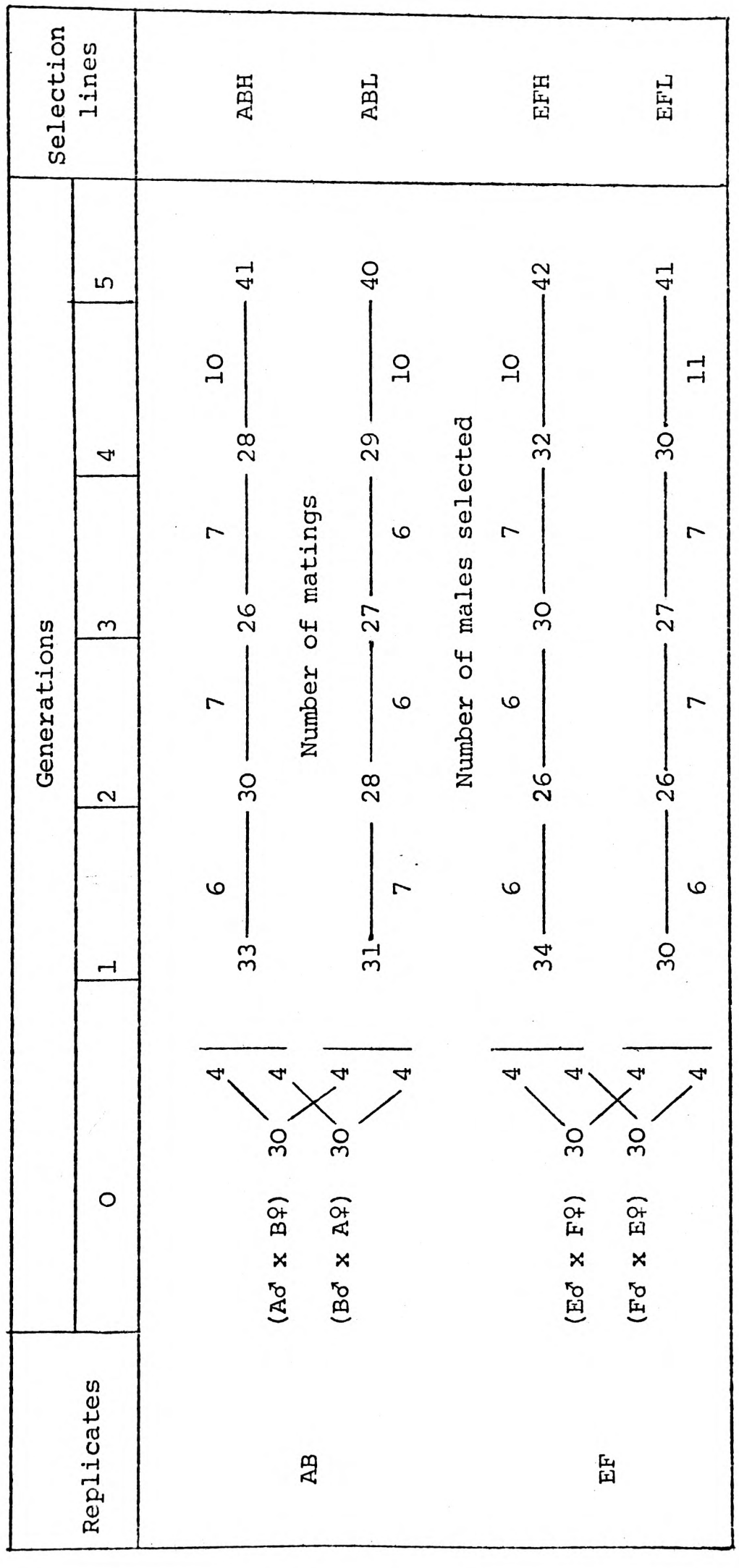
In the initial generation (O), the males and the females of A and B Control lines were reciprocally mated (A males x B females and B males x A females) and the same procedure was followed for E and F individuals (E males x F females and F males x E females). The male mice were scored for testes weights after sacrificing. Four high scored and four low scored males were selected on the basis of their testes weights out of 30 males recorded in each reciprocal cross. The progeny of A and B males were represented equally in the high (ABH) and low (ABL) lines of the AB replicate and so were the progeny of E and F males in the high (EFH) and the low (EFL) lines in the EF replicates. In subsequent generations each selection line was maintained by mating inter se. The experiment was continued up to five generations of selection. In the first generation, the population consisted of 8 full sib families in each line. The number of individuals recorded and the number of males selected in each generation are summarised in Fig. 2.1.

(iii) Measuring the traits

(a) Testis weight

To take the testes weights of individual mice required sacrificing of males and so progeny were obtained from the males before scoring for testis weight. The males were therefore allowed to mate with contemporary females at 9 weeks of age and they were scored for testis weight

Fig. 2.1. Number of individuals recorded and of males selected in each generation.



at 11 weeks of age, after two weeks of pairing when the females were assumed to be pregnant and the males to be mature. The weights of each of the two testes from individual males were recorded in milligrams.

(b) Body weight

Body weights of individual males and females were recorded in each generation at 6 weeks of age and at 9 weeks of age, i.e. at the time of mating. Live weights of males were taken at 11 weeks of age just before scoring for testis weight and that of females at approximately 18 weeks of age before scoring for ovulation rate.

(c) Litter size

Litter size at birth, (the number of young born alive in the first litter) and at weaning (the number of young successfully weaned by individual females) were recorded in each generation. Individual females were allowed to rear their whole litter without any adjustment to a standard number. The litter was weaned approximately 3 weeks after birth.

(d) Ovulation rate

Ovulation rate in primiparous females (females which have given birth to one litter) was recorded in the second, third and fourth generation of the selected lines. The females were scored for ovulation rate at approximately 18 weeks of age, about two weeks after weaning their progeny. Ovulation rate in nulliparous females (females that had not been mated or borne a litter) was measured in the fifth generation of selected lines. The number of females scored for ovulation rate in different generations of the selected lines are given in Table 2.1.

Table 2.1. The number of females scored for ovulation rate (generations 2 to 4 primiparous, generation 5 nulliparous).

Selection lines	Generations			
	2	3	4	5
ABH	30	25	24	41
ABL	26	25	28	40
EFH	25	27	29	42
EFL	25	27	28	41

The techniques described in Land and Falconer (1969) for counting the number of ovulated eggs in mice were followed. Natural ovulation in mice occurs during the night close to the time of mating. Females paired with males were examined each morning and the occurrence of ovulation was identified by the presence of a copulatory plug in the vagina. The excess contemporary males of each line in each generation were used for mating and detecting the plug. The females were dissected after the identification of plugs before 13.00 hours of the same day. The fallopian tube was removed carefully and examined under the dissecting microscope. The presence of eggs in the fallopian tube which are embedded in cumulus cells can be identified as a discrete swelling; and the rupture of the fallopian tube at that point enables the eggs to be removed. The eggs were identified in the cumulus and counted. The eggs counted from the right and the left tubes for each individual were pooled together.

(iv) Symbols and abbreviations used.

T	Testis weight
O	Ovulation rate
W	Body weight
M	Number of males selected
M*	Number of males measured
F	Number of female parents
F*	Number of females measured
N	Total number of individuals recorded.
R	Response to selection
CR	Correlated response
σ^2	Phenotypic variance
σ^2_A	Additive genetic variance
b	Phenotypic regression
b_A	Genetic regression
r	Phenotypic correlation
r_A	Genetic correlation
V	Variance
COV	Covariance
d	Drift
e	Error
h^2	Heritability
i	Intensity of selection
p	Proportion selected
s	Selection differential
t	Number of generations
Lm,Lf	Generation interval in male, in female.
ΔG	Rate of genetic gain.

(v) A Study of the phenotypic relationship between body weight and testis weight in the Q strain.

The phenotypic relationship between body weight and testis weight was studied before the selection experiment began to decide whether testis weight should be corrected for body weight when used as a selection criterion. Initially, a total of 124 males were taken from the A, C, D, E and F Control lines of the 38th generation. The body weights and the testis weights were recorded at 10 weeks of age. A total of 120 males recorded later at 11 weeks of age in the A, B, E and F Control lines of the 39th generation for starting the selection experiment were also included in this study. The correlation coefficients between body weight and testis weight and the regression coefficients of testis weight on body weight within each Control lines in different generations are given in Table 2.2. In the 38th generation, a statistically significant negative relationship was observed in A but significant positive relationships were observed in D and E. Analyses of regression showed that the slopes differed significantly between lines. In the 39th generation, the relationship was found to be significantly positive in A. The relationship in F was found to be positive in the 38th generation but negative in the 39th generation; neither of them was statistically significant. As there were no consistent relationships between body weight and testis weight in different lines and in different generations, it was decided to avoid correction on testis weights for body weights in selecting mice.

The data collected in different control lines of the Q strain were pooled together and used to calculate some of the phenotypic parameters of body weight and testis weight in mice. Estimates of phenotypic parameters are given in Table 2.3. It was noted that the phenotypic

Table 2.2. The mean body weights and testis weights and the correlation and regression between body weight (W) and testis weight (T) in different Control lines of the Q strain.

	Control lines						
	A	B	C	D	E	F	Units
n	19		13	34	27	31	*
$\bar{W}$	31.87 ± 0.61	-	28.81 ± 0.79	28.13 ± 0.66	31.03 ± 0.45	27.66 ± 0.36	g
$\bar{T}$	203.60 ± 5.75	-	249.00 ± 9.84	213.20 ± 5.14	173.00 ± 6.45	194.80 ± 3.99	mg
r	-0.44 ± 0.22		0.25 ± 0.29	0.55 ± 0.15	0.41 ± 0.18	0.09 ± 0.18	
b_{TW}	-4.10 ± 2.05		3.00 ± 3.6	4.10 ± 1.15	5.90 ± 2.64	0.90 ± 2.06	mg/g
n	30	30	-	-	30	30	**
$\bar{W}$	26.85 ± 0.54	30.43 ± 0.33			28.44 ± 0.42	25.97 ± 0.36	g
$\bar{T}$	183.03 ± 7.33	199.97 ± 5.55			183.87 ± 6.52	198.76 ± 5.44	mg
r	0.61 ± 0.15	0.29 ± 0.18			0.34 ± 0.18	-0.11 ± 0.19	
b_{TW}	8.20 ± 2.03	4.80 ± 2.79			5.60 ± 1.93	-1.37 ± 1.25	mg/g

* Recorded in the 38th generation.

** Recorded in the 39th generation.

Table 2.3. Phenotypic parameters of testis weights and body weight in the Q strain.

(a) Pooled within lines A, C, D, E and F in the 38th generation. (N = 124)

	Testis Weight	Body Weight	Between Testis Weight and Body Weight
Mean	206.70 mg	29.30 g	$r(TW) = 0.29 \pm 0.09$
Variance	840.34 mg^2	8.21 g^2	
Standard deviation	28.99 mg	2.87 g	$b_{TW} = 2.9 \pm 0.90$ mg/g
S.d. of mean	2.60	0.26 g	
Coeff. of Variation	0.14	0.10	$b_{WT} = 0.028 \pm 0.009$ g/mg

(b) Pooled within lines of A, B, E and F in the 39th generation (N = 120).

Mean	191.41	27.92	$r(TW) = 0.34 \pm 0.09$
Variance	1147.41	5.50	
Standard deviation	33.87	2.35	$b_{TW} = 4.96 \pm 1.27$
S.d. of mean	3.09	0.21	
Coeff. of Variation	0.18	8.42	$b_{WT} = 0.024 \pm 0.006$

(c) Pooled ignoring lines and generations (N = 244).

Mean	197.85	28.62	$r(TW) = 0.20 + 0.06$
Variance	1321.08	9.86	
Standard deviation	36.35	3.14	$b_{TW} = 2.34 \pm 0.73$
S.d. of mean	2.33	0.20	
Coeff. of Variation	0.18	0.11	$b_{WT} = 0.018 \pm 0.005$

coefficient of variation of testis weight was about 50% greater than that of body weight at the same age and the phenotypic correlation between body weight and testis weight was low.

III. RESULTS

(i) The response to selection for testis weight

The response to selection in five generations of individual selection indicated that the selection was effective in separating the high and the low lines and was fairly repeatable in both replicates. The mean testis weights of high and low lines in both replicates are shown in Fig. 2.2a. The mean testis weights, selection differentials and responses in each generation along with cumulative selection differentials and responses are given in Table 2.4. There were clear increases in testis weight in the high lines and decreases in the low lines. The mean testis weight in the base population was 191 mg. On average, testis weights increased by 54 mg in the high lines and decreased by 58 mg in the low lines in the fifth generation. As there was no Control line for comparing systematic increase or decrease of testis weights in the selected lines, the response to selection was measured in terms of the divergence between the high and the low lines. The divergences of testis weights in the fifth generation were 114 ± 18 mg and 110 ± 18 mg in AB and EF, respectively. On average, the divergence was 112 ± 13 mg which was about 60% of the base population mean. An approximate standard deviation of divergence was calculated from the square root of the expected variance of divergence and the variance was obtained by:

$$V(Rt) = 2\sigma^2\left[\frac{th^2(1-h^2)}{4M} + \frac{th^4}{4M^*} + \frac{th^2}{4F} + \frac{1-\frac{3}{2}h^4}{M^*}\right]$$

where M is the number of males selected, M* is the number of males

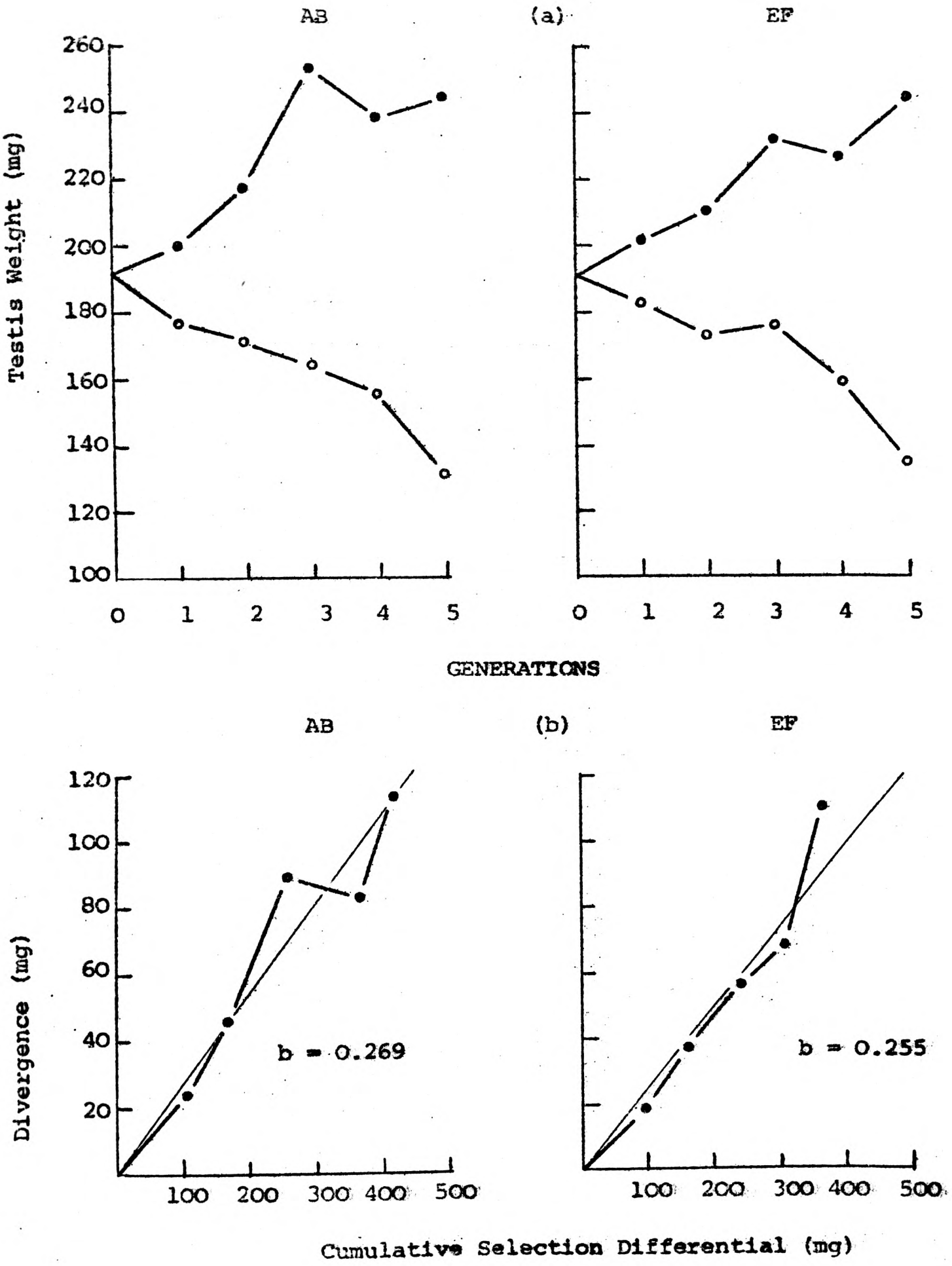


Fig. 2.2 (a) Response to selection for testis weight in high (solid circle) and low (open circle) lines in AB and EF. Generation means plotted against generations of selection.

(b) Divergence of high and low lines in AB and EF plotted against cumulative selection differential. Straight lines are the linear regressions in AB and EF.

Table 2.4. Response to selection for testis weight (mg).

Generation	ABH			ABL			Cumulated sel. dif.	(ABH-ABL) Cum. Response	S.E.* of Mean diff.
	Mean ± s.e.*	Selection differential:	Response	Mean ± s.e.*	Selection differential	Response			
0	191.50 ± 4.69	53.26	-	191.50 ± 4.69	53.56	-	106.82	-	
1	200.24 ± 4.28	30.60	8.74	176.85 ± 4.45	29.69	14.65	167.11	23.39	6.12
2	217.51 ± 7.17	56.13	17.27	171.61 ± 6.37	42.08	5.24	256.32	45.90	9.59
3	253.31 ± 13.18	62.77	35.80	164.21 ± 6.46	46.26	7.40	365.35	89.10	14.68
4	239.36 ± 5.35	22.80	-13.95	156.11 ± 5.59	29.37	8.10	417.52	83.25	7.74
5	245.54 ± 4.98	-	6.18	131.52 ± 5.63	-	24.59	-	114.02	7.52
	Average	45.11	10.81		40.19	12.00			
	EFH			EFL				(EFH-EFL)	
0	190.88 ± 4.37	40.45	-	190.88 ± 4.37	58.23	-	98.68	-	
1	201.40 ± 4.01	37.20	10.52	182.67 ± 5.51	26.57	8.21	162.45	18.73	.81
2	210.24 ± 7.32	51.75	8.84	172.91 ± 5.51	28.17	9.76	242.37	37.33	9.16
3	231.97 ± 6.48	39.88	21.73	176.19 ± 7.25	27.70	-3.28	309.95	55.78	9.72
4	227.48 ± 5.71	35.91	-4.49	159.05 ± 5.00	22.17	17.14	368.03	68.43	7.60
5	245.22 ± 4.30	-	17.74	135.12 ± 5.02	-	23.93	-	110.10	6.61
	Average	41.04	10.87		32.57	11.55			

* S.E.'s from within generation mean, not corrected for drift.

recorded and F is the number of female parents. The value of heritability of testis weights used in this formulae is given later.

The divergence of high and low lines was plotted against the cumulative selection differentials and is shown in Fig. 2.2b. The regression line was passed through the origin. The regression coefficients were 0.269 $\pm$ 0.046 and 0.255 $\pm$ 0.046 in AB and EF, respectively. As there was no significant difference between the two estimates, they were pooled together and the pooled estimate of regression coefficient was 0.263 $\pm$ 0.033. An approximation of standard error for the observed regression coefficient was made from the expected variance of regression of response on cumulative selection differential in generations selected. The formulae used for calculating the variance of regression was taken from Hill (1972) and is given below:

$$V(bc) = \frac{\sigma}{s^2 t(t+1)(2t+1)} \left[\frac{2t^2+2t+1}{5}\sigma^2 d + \sigma^2 e + \frac{3t(t+1)}{2(2t+1)} h^2 \sigma e^2\right]$$

The drift (d) and the error (e) variances were estimated from the formulae for variance of response. Average cumulative selection differentials (s) was used in this formulae, assuming that selection differential was constant in all generations.

Since the selection was done in one sex, heritability would be twice as much as the observed value of regression coefficient. Hence the estimates of heritability of testis weights were 0.540 $\pm$ 0.092 in AB and 0.510 $\pm$ 0.092 in EF and the pooled estimate was 0.520 $\pm$ 0.065.

(ii) Correlated response in ovulation rate

The mean ovulation rates of primiparous females measured in the second, third and fourth generation and the mean ovulation rates of nulliparous females measured in the fifth generation of the selected lines

are shown in Fig. 2.3a. Correlated response was measured from the divergence of ovulation rate between the selected high and low lines. The divergence between the high and the low lines along with the mean ovulation rates and the mean body weights at the time of scoring for each line in each generation are given in Table 2.5.

To estimate the genetic regression of primiparous ovulation rate on testis weight, the divergence of ovulation rate (correlated response) was plotted against the divergence of testis weights (direct response) and is shown in Fig. 2.3b. The regression line was passed through the origin. Since the correlated response $CR_O = b_{(A)OT} R_T$ (see for example, Falconer, 1960b) hence $b_{(A)OT} = CR_O/R_T$, where CR_O is the correlated response of trait O (ovulation rate) from direct selection of trait T (testis weight), $b_{(A)OT}$ is the genetic regression of O on T, and R_T is the direct response in trait T. The genetic regression of nulliparous ovulation rate on testis weight was calculated from the ratio of divergence of ovulation rate to the divergence of testis weight ($b_{(A)OT} = CR_O/R_T$) in the fifth generation.

The observed correlated responses in primiparous ovulation rate in the fourth generation and that in nulliparous ovulation rate in the fifth generation and the estimated genetic regressions of ovulation rate on testis weight in AB and EF are given in Table 2.6. The separate estimates in AB and EF were pooled together and the pooled estimates are also given in the same table.

Approximate standard errors for correlated response were calculated from the expected variance of correlated response (the formula given in Hill, 1971) and the variance was obtained by:

$$V(CR_O) = 2\sigma^2_O \left[\frac{th^2_O(1-h^2_T r^2_{(A)OT}(1-p))}{4M} + \frac{th^2_O}{4F} + \frac{1}{F^*}\right]$$

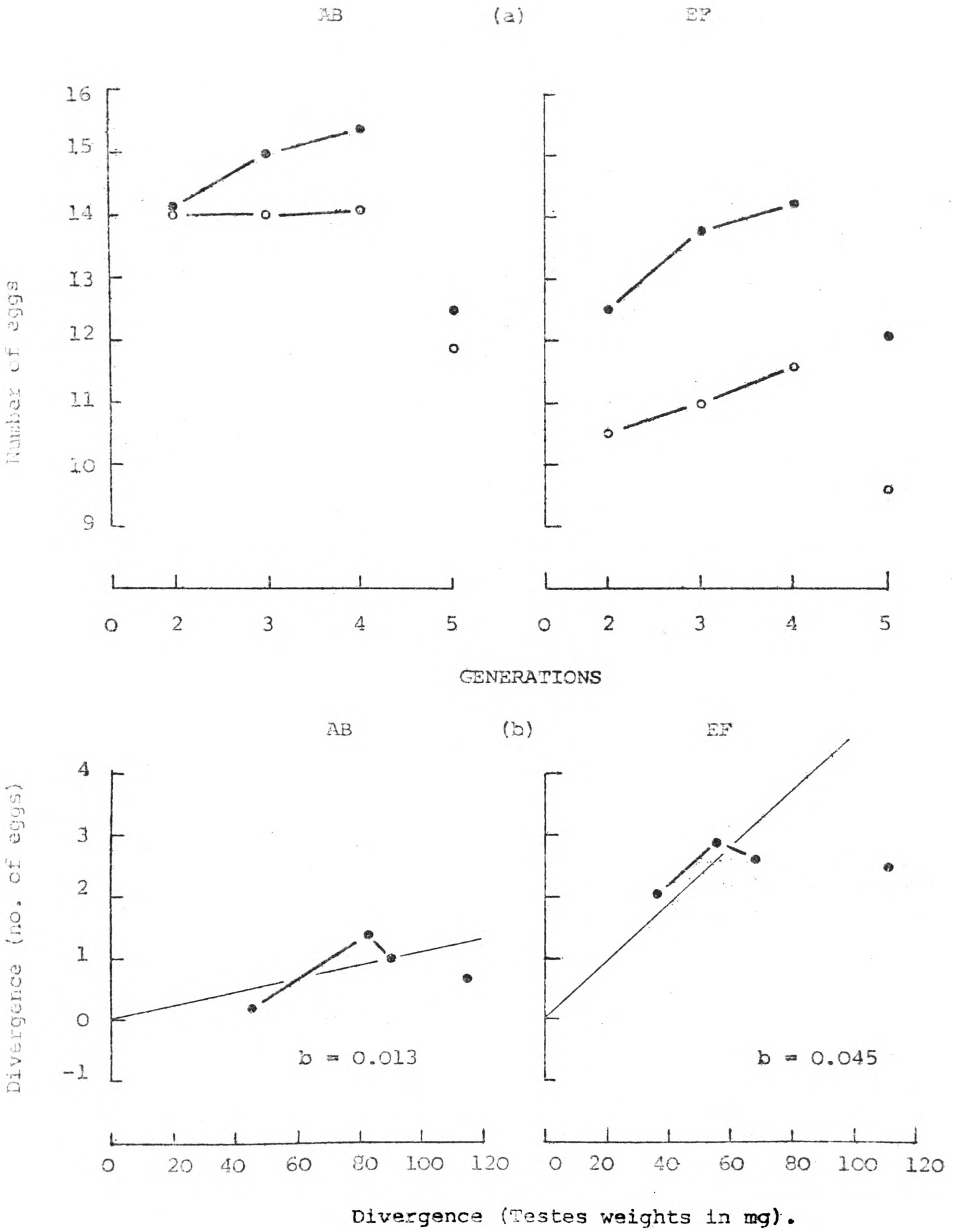


Fig. 2.3 (a) The ovulation rate of primiparous (in 2nd, 3rd and 4th generations) and nulliparous females (in 5th generation) of the selected high (solid circle) and low (open circle) lines in AB and EF.

(b) The divergence of ovulation rate between the high and the low lines (correlated response) in AB and EF plotted against the divergence of testis weight (direct response). Straight lines are the linear regressions in AB and EF.

Table 2.5. Ovulation rates and body weights at the time of scoring of the selected lines.
(Primiparous in generations 2, 3 and 4 and nulliparous in generation 5)

Generation	ABH		ABL		ABH-ABL Ovulation rate	S.E.* of mean diff.
	Body Weights (g) Mean ± s.e.*	Ovulation rate Mean ± s.e.*	Body Weights (g) Mean ± s.e.*	Ovulation rate Mean ± s.e.		
2	28.64 ± 0.47	14.13 ± 0.36	29.35 ± 0.42	13.96 ± 0.61	0.17	.068
3	30.34 ± 0.58	15.00 ± 0.44	30.06 ± 0.46	14.00 ± 0.51	1.00	.068
4	29.99 ± 0.43	15.42 ± 0.37	29.35 ± 0.38	14.07 ± 0.34	1.35	0.50
5	23.12 ± 0.28	12.56 ± 0.34	22.68 ± 0.27	11.96 ± 0.30	0.66	0.46
	EFH		EFL		EFH-EFL	
2	28.30 ± 0.46	12.52 ± 0.47	25.92 ± 0.53	10.48 ± 0.59	2.04	0.76
3	29.17 ± 0.34	13.81 ± 0.41	27.21 ± 0.46	10.96 ± 0.42	2.85	0.59
4	28.53 ± 0.37	14.21 ± 0.30	26.14 ± 0.30	11.57 ± 0.36	2.64	0.47
5	21.70 ± 0.31	12.10 ± 0.33	19.65 ± 0.24	9.63 ± 0.36	2.47	0.49

* S.E.'s from within generation means, not corrected for drift.

where M is the number of males selected in each line, F is the number of female parents, F* is the number of females recorded and p is the proportion selected. Accordingly, standard errors for the estimated genetic regressions were calculated from the expected variance of genetic regression and the variance was obtained by:

$$V(b_A) = b_A^2 \left[\frac{V(CR_O)}{CR_O^2} + \frac{V(R_T)}{R_T^2} - 2\,\frac{Cov(CR_O, R_T)}{CR_O\, R_T}\right]$$

where b_A is the observed genetic regression, CR_O is the correlated response in ovulation rate and R_T is the direct response in testis weight.

Table 2.6. Correlated response in ovulation rate and the genetic regression of ovulation rate on testis weight.

Replicate	Correlated response (No. of eggs)		Genetic regression (No. of eggs per mg.)	
	Primiparous	Nulliparous	Primiparous	Nulliparous
AB	1.35 ± 1.07	0.66 ± 0.93	0.013 ± 0.010	0.006 ± 0.008
EF	2.64 ± 1.07	2.45 ± 0.93	0.045 ± 0.018	0.022 ± 0.008
Pooled (Within replicates)	2.00 ± 0.76	1.52 ± 0.66	0.029 ± 0.011	0.014 ± 0.007

(iii) Correlated response in litter size

The mean litter sizes at birth (number of young born alive in the first litter) and at weaning (number of young successfully reared up to 3 weeks of age by individual females) produced by the females in each generation in the different selected lines are shown in Fig. 2.4a. The mean litter sizes at weaning followed more or less the same pattern of

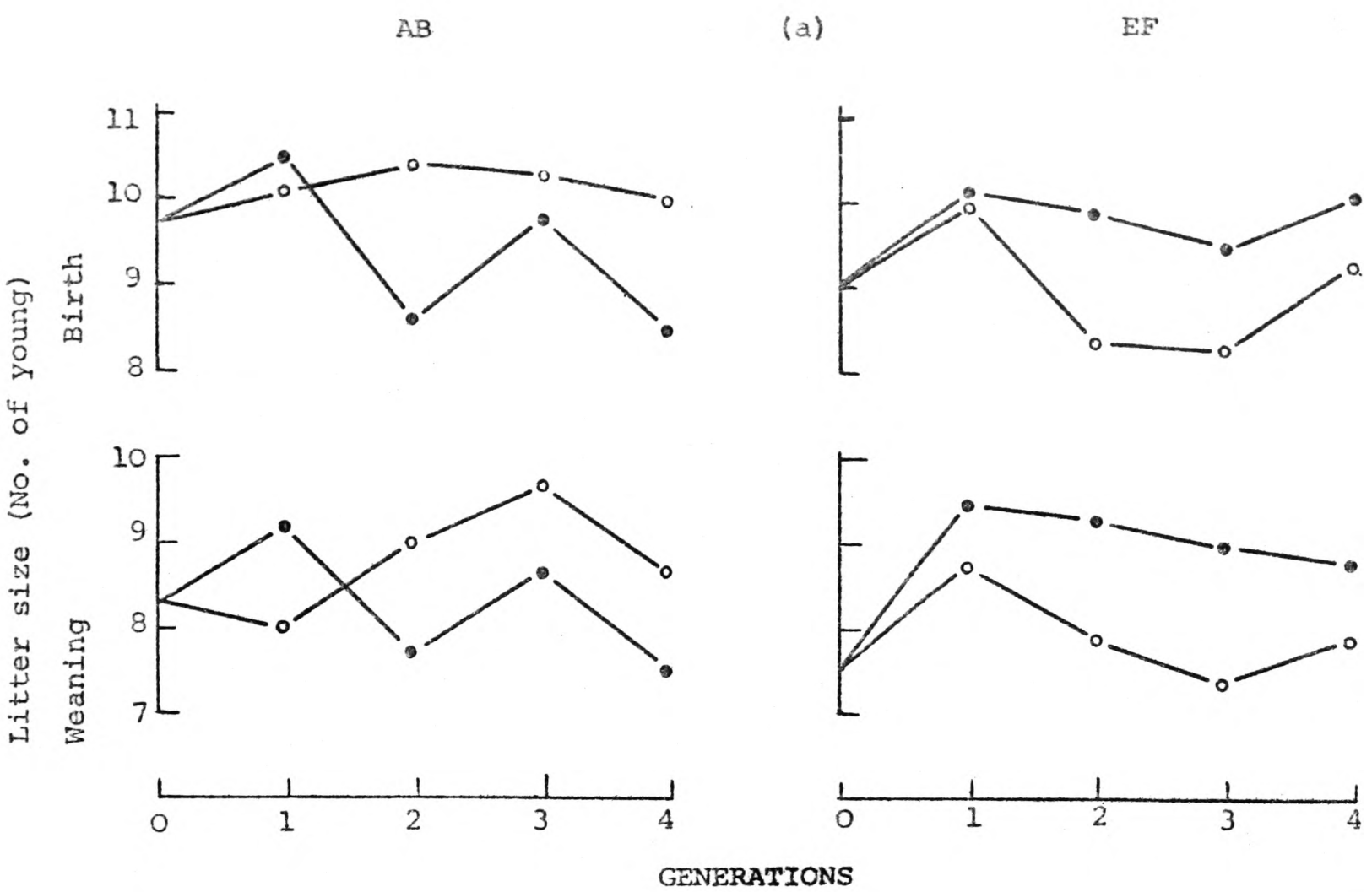


Fig. 2.4 (a) Litter size at birth and at weaning of high (solid circle) and low (open circle) lines in AB and EF.

(b) Divergence of litter size at birth (correlated response) in AB and EF plotted against the divergence of testis weights (direct response).

Table 2.7. Average litter size at birth and at weaning (number of young).

Generation	ABH		ABL			
	Litter size at birth	Litter size at weaning.	Litter size at birth	Litter size at weaning.	ABH-ABL at birth.	ABH-ABL at weaning.
0	9.76 ± 0.32	8.34	9.76 ± 0.32	8.34	-	-
1	10.48 ± 0.31	9.19	10.06 ± 0.43	7.97	0.42 ± 0.52	1.22
2	8.66 ± 0.44	7.72	10.36 ± 0.33	9.00	-1.70 ± 0.56	-1.28
3	9.83 ± 0.68	8.74	10.33 ± 0.37	9.70	-0.50 ± 0.77	-0.96
4	8.52 ± 0.59	7.52	10.04 ± 0.41	8.74	-1.52 ± 0.72	-1.22
	EFH		EFL		EFH-EFL	EFH-EFL
0	9.05 ± 0.31	7.47	9.05 ± 0.31	7.47	-	-
1	10.15 ± 0.33	9.45	9.97 ± 0.32	8.67	0.18 ± 0.46	0.78
2	9.92 ± 0.38	9.31	8.36 ± 0.70	7.92	1.56 ± 0.79	1.39
3	9.48 ± 0.49	9.00	8.27 ± 0.43	7.42	1.21 ± 0.65	1.58
4	10.06 ± 0.34	8.84	9.31 ± 0.41	7.86	0.75 ± 0.53	0.98

* ± S.E.'s from within generation mean, not corrected for drift.

fluctuations between generations as that observed in litter size at birth. The divergence of litter size at birth between the high and the low lines plotted against the divergence of testis weights are shown in Fig. 2.4b. The regression of litter size on testis weight was found to be negative in AB (-0.0138 young per mg) and positive in EF (0.0208 young per mg). The mean litter sizes at birth and at weaning and their deviations between the high and the low lines in different generations are given in Table 2.7.

Although a positive correlated response in litter size occurred in EF, there was a great difference between the replicates and on average, correlated response in litter size appeared to be nil. Considering the ovulation rates in different selected lines, it seemed that the number of eggs lost (an account of unfertilized eggs and embryonic mortality) was higher in high lines in which the ovulation rate was higher and lower in the low lines in which the ovulation rate was lower. The percentage of lost eggs estimated from the ovulation rate of nulliparous females in the fifth generation and litter size in the fourth generation in different selected lines are given in Table 2.8.

Table 2.8. Percentages of lost eggs in different selected lines.

% Lost Eggs (unfertilized eggs + embryonic mortality)				Deviation (High - Low)
ABH	32	ABL	16	16
EFH	17	EFL	4	13

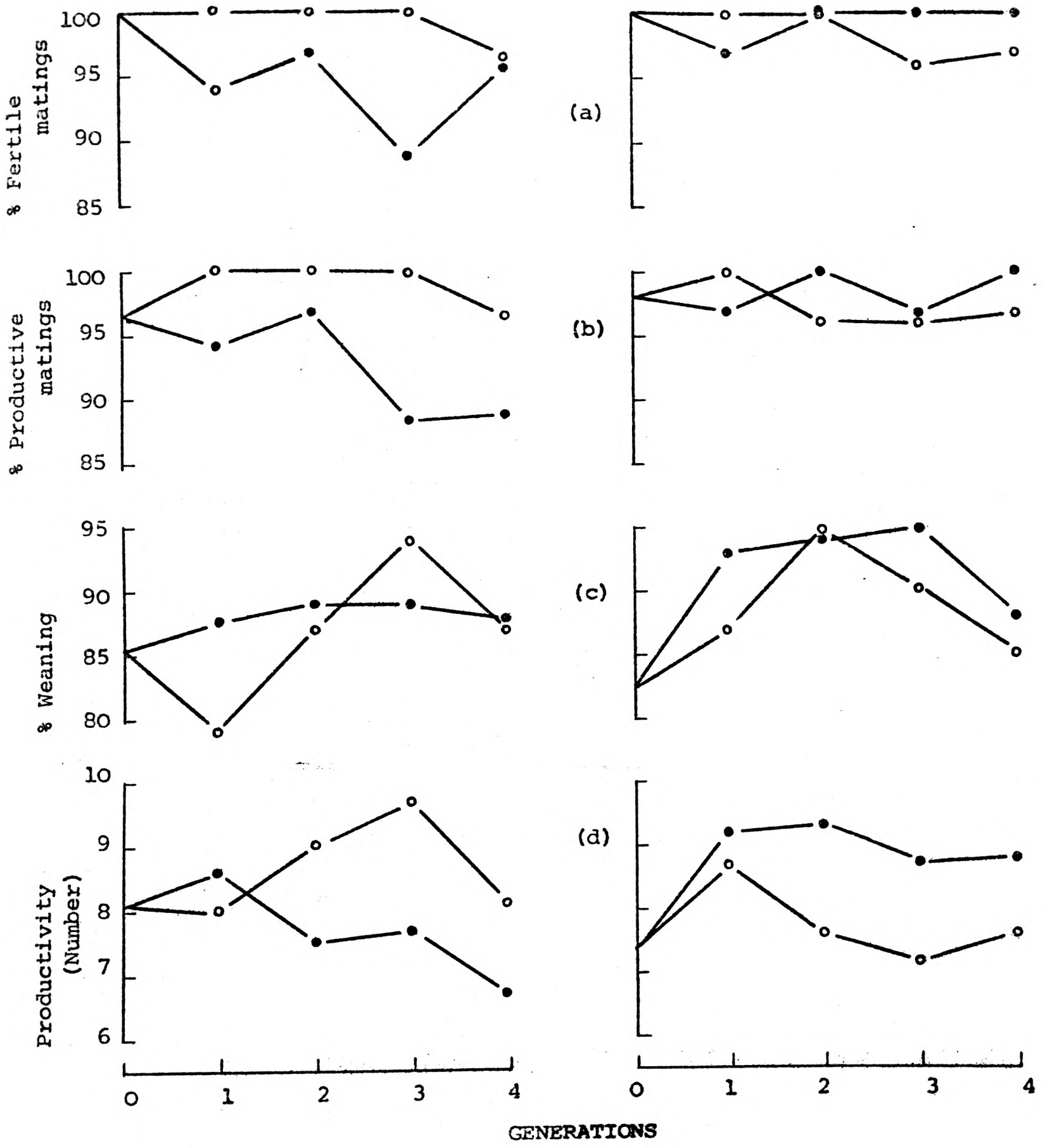


Fig. 2.5 The productive performance of the lines selected for high (solid circle) and low (open circle) testis weights in AB and EF.

(a) Fertile matings - matings that did cause pregnancy.
(b) Productive matings - matings that produced one or more live young at birth.
(c) % weaning - the proportion surviving to weaning of those born alive.
(d) Productivity - the number of young weaned per mating made.

Table 2.9. Components of productivity in different selected lines.

Gener-ations	Selection lines	No. of Matings	% Fertile Mating	% Productive Mating	No. of young produced.	% weaned	No. of young weaned/mating
0	AB	60	100 (60)	97 (58)	566	85.5 (484)	8.1
	EF	60	100 (60)	98 (59)	532	82.5 (441)	7.4
1	ABH	33	94 (31)	94 (31)	325	87.7 (285)	8.6
	ABL	31	100 (31)	100 (31)	332	79.2 (247)	8.0
	EFH	34	97 (33)	97 (33)	335	93.1 (312)	9.2
	EFL	30	100 (30)	100 (30)	299	87.0 (260)	8.7
2	ABH	30	97 (29)	97 (29)	251	89.2 (224)	7.5
	ABL	28	100 (28)	100 (28)	290	86.7 (252)	9.0
	EFH	26	100 (26)	100 (26)	258	93.8 (242)	9.3
	EFL	26	100 (26)	96 (25)	209	94.7 (198)	7.6
3	ABH	26	88 (23)	88 (23)	226	88.9 (201)	7.7
	ABL	27	100 (27)	100 (27)	279	93.9 (262)	9.7
	EFH	30	100 (30)	97 (29)	275	94.9 (261)	8.7
	EFL	27	96 (26)	96 (26)	215	89.8 (193)	7.1
4	ABH	28	96 (27)	89 (25)	213	88.3 (188)	6.7
	ABL	29	97 (28)	97 (28)	281	84.0 (236)	8.1
	EFH	32	100 (32)	100 (32)	322	87.9 (283)	8.8
	EFL	30	97 (29)	97 (29)	269	84.8 (228)	7.6

Number of fertile matings, productive matings and young weaned are given in parenthesis.

(iv) Correlated response in productivity

The productivity of the mated pairs was measured in terms of: (a) the proportion of fertile matings, i.e. matings that caused pregnancy, (b) the proportion of productive matings, i.e. matings that produced one or more live young, which excluded the infertile matings, difficulty in parturition, litter born dead, and litter born alive or dead, but all young eaten up by their mother, (c) weaning rate, i.e. the proportion of young born alive surviving to weaning, and (d) productive number, i.e. the number of young weaned per mating made. The different components of productivity observed in the different selected lines are shown in Fig. 2.5.

The apparent decrease in percent fertile mating in the ABH line might be due to chance because the number of infertile matings varied from 0 to 3 in each selection line (Table 2.9). The weaning rate was approximately the same in the high and the low lines, on average the high line was slightly better. The number of young weaned per mating, showed more or less the same type of picture as observed in the litter sizes at weaning.

(v) Correlated response in body weight

The mean body weights of males and females at 6 weeks of age, females at 9 weeks of age (i.e. at the time of mating) and males at 11 weeks of age (i.e. at the time of scoring for testis weight) are shown in Fig. 2.6. There was little difference between the mean body weights of high and low lines in AB but considerable difference was observed between the mean body weights of high and low lines in EF at different ages. In general, the trend of change in body weight at 6, 9 and 11 weeks of age was similar in each replicate. The mean body weights in

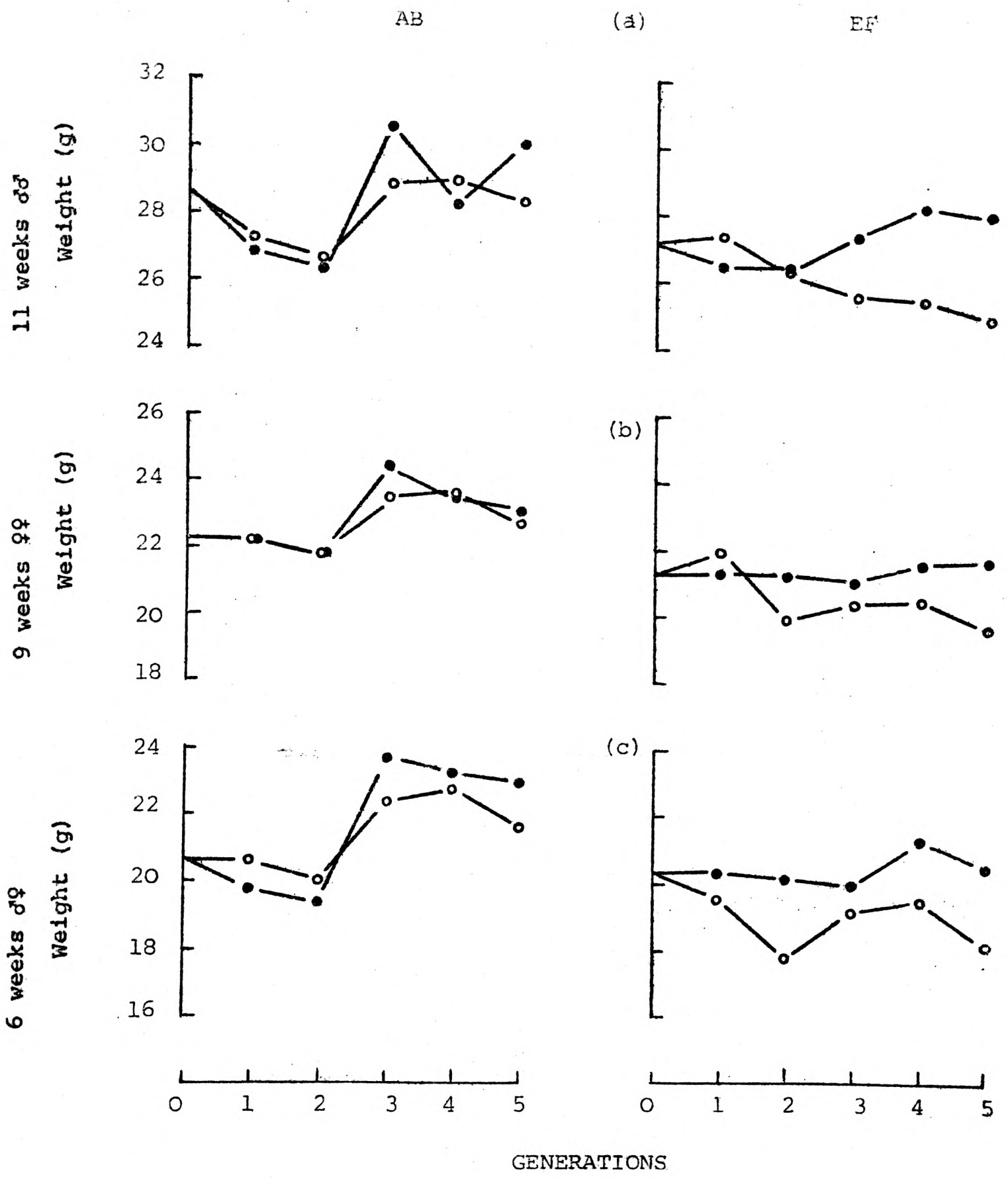


Fig. 2.6 The body weights of the lines selected for high (solid circle) and low (open circle) testis weights in AB and EF.

(a) Males at 11 weeks, i.e. at the time of scoring for testis weights.
(b) Females at 9 weeks, i.e. at the time of mating.
(c) Combined males and females at 6 weeks of age.

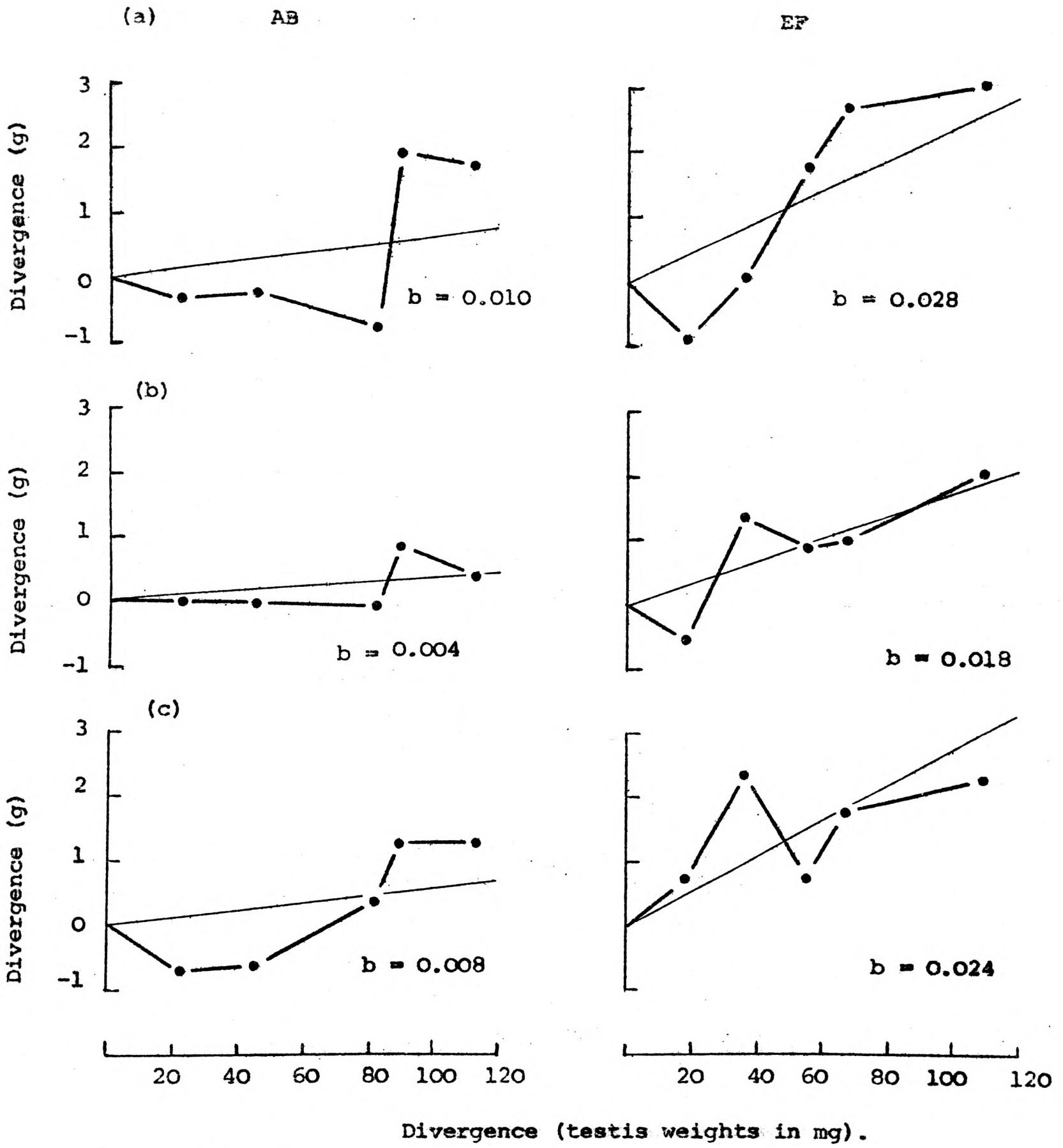


Fig. 2.7. Divergence of body weights (correlated response) in AB and EF plotted against the divergence of testis weights (direct response).

(a) Males at 11 weeks of age.
(b) Females at 9 weeks of age.
(c) Combined males and females at 6 weeks of age.

Table 2.10. Average body weights (g) of the selected lines.

Generation	ABH			ABL			Deviations		
	6 wks. wt. males and females	Mating wt. (9 wks) Females	Scoring wt. (11 weeks) Males	6 wks. wt. males and females	Mating wt. (9 wks.) females	Scoring wt. (11 weeks) males	ABH-ABL 6 weeks	ABH-ABL 9 weeks	ABH-ABL 11 weeks
0	20.73	22.34	28.64	20.73	22.34	28.64	-	-	-
1	19.80	22.13	26.79	20.58	22.14	27.12	-0.78	-0.01	-0.33
2	19.34	21.84	26.32	19.98	21.84	26.55	-0.64	0.00	-0.23
3	23.71	24.41	30.63	22.38	23.50	28.84	1.33	0.91	1.79
4	23.17	23.52	28.30	22.78	23.57	28.99	0.39	-0.05	-0.69
5	22.90	23.13	30.10	21.63	22.68	28.27	1.27	0.45	1.83
	EFH			EFL			EFH-EFL	EFH-EFL	EFH-EFL
0	20.29	21.34	27.18	20.29	21.34	27.18	-	-	-
1	20.24	21.29	26.38	19.50	21.86	27.28	0.74	-0.57	-0.90
2	20.07	21.30	26.30	17.72	19.86	26.22	2.35	1.43	0.08
3	19.89	21.04	27.42	19.14	20.15	25.61	0.75	0.89	1.81
4	21.34	21.56	28.22	19.54	20.52	25.51	1.80	1.04	2.71
5	20.46	21.70	28.00	18.13	19.65	24.92	2.33	2.05	3.08

S.E. of Means ranged from 0.30 to 0.65

Table 2.11. Correlated response in body weight (CRW) and genetic regression of body weight on testis weight (b(A)WT) at different ages.

AGE	Replicates				Pooled within replicates	
	AB		EF			
	CRW (g)	b(A)WT (g/mg)	CRW (g)	b(A)WT (g/mg)	CRW (g)	b(A)WT (g/mg)
6 weeks	1.27 ± 1.45	0.008 ± 0.006	2.33 ± 1.45	0.024 ± 0.019	1.80 ± 1.03	0.016 ± 0.009
9 weeks	0.45 ± 1.54	0.004 ± 0.003	2.05 ± 1.54	0.018 ± 0.014	1.25 ± 1.09	0.011 ± 0.006
11 weeks	1.83 ± 1.54	0.010 ± 0.008	3.08 ± 1.54	0.028 ± 0.022	2.45 ± 1.09	0.019 ± 0.011

different selected lines along with the divergence between the high and the low lines are given in Table 2.10.

The divergence of body weight between the high and the low lines (correlated response) is plotted against the divergence of testis weight (direct response) in Fig. 2.7. The genetic regression of body weight on testis weight ($b_{(A)WT} = CR_W/R_T$) at different ages was estimated from the regression of divergence in body weight on the divergence in testis weight. The observed correlated response in body weight in the fifth generation and the estimated genetic regression of body weight on testis weight are given in Table 2.11.

Approximate standard errors of the observed correlated response and the estimated genetic regression (shown in Table 2.11) were calculated from the expected variances. The variances of correlated response and genetic regression were obtained by the same way as for ovulation rate.

IV. DISCUSSION AND CONCLUSIONS

One of the aims of this experiment was to observe how much change in testis size could be brought about by selection and how much additive genetic variance existed in the population. The results indicated that a considerable amount of response occurred up to five generations of selection, equivalent to about three standard deviations of testis size. The realised heritability of testis size was found to be high, around 50%. Although there was no report for the magnitude of heritability in the literature, Shire and Barte (1972) reported that much of the differences in testis size in mice was genetic in origin.

Statistically significant correlated response ($p < 0.05$) occurred in ovulation rates of both primiparous and nulliparous females. The results suggest that ovulation rate and testis size are genetically

correlated. This observation was in agreement with the observation of Land (1973) who reported that a correlated change in testis size occurred due to selection for ovulation rate in mice. The estimates of genetic regression of ovulation rate on testis weight indicated that the change in ovulation rate was about 3 eggs in primiparous and 1.5 eggs in nulliparous females per 100 mg of change in testis weight. How the selection for testis size contributed to the change in ovulation rate is a question of debate. It is postulated that the growth of testis and its size are mainly dependent on the level and activity of circulating gonadotrophic hormones and the sensitivity of the testis to these hormones. Therefore, the change in testis size by genetic selection should simultaneously change these components and should reflect on components of fertility in the female through ovarian activity as an effect of change in level and activity of gonadotrophins. According to this hypothesis, it is probable that the change in ovulation rate occurred due to the change in level and activity of gonadotrophic hormones and sensitivity of ovary to these hormones. Tests for testis and ovarian sensitivity to gonadotrophins or the measurements of activity of gonadotrophins were not attempted in the present experiment because they require complicated procedures and there are difficulties in obtaining accurate measurements in small mammals like the mouse. It was therefore not possible to identify and isolate the specific physiological reason for correlated change in ovulation rate. However, Land and Falconer (1969) reported that the selection for ovulation rate led to different physiological changes in the two directions of selection; upward selection led primarily to increased gonadotrophin activity, while downward selection led to reduced ovarian sensitivity. This conclusion was drawn from measurements of ovarian

sensitivity to gonadotraphins in the selected lines. It was also reported that there were positive genetic relationships between ovulation rate and ovarian sensitivity and between body weight and ovarian sensitivity (Land, 1970c).

An attempt was made to estimate the genetic correlations between ovulation rate and testis weight and between body weight and testis weight. Some of the relevant information was taken from other experiments which were carried out in the Q strain. The partial genetic correlation between ovulation rate and testis weight and the partial genetic regression of ovulation rate on testis weight for constant body weight were also estimated. The procedures for calculating these parameters and the observed results are given here.

The genetic correlation between ovulation rate and testis weight was calculated from the observed genetic regression of ovulation rate (pooled within replicates) multiplied by the ratio of genetic standard deviations ($r_{(A)OT} = b_{(A)OT} \cdot \sigma_{(A)T}/\sigma_{(A)O}$). The value of the genetic standard deviation of testis weight (22 mg) was obtained from the present experiment and the value of the genetic standard deviation of ovulation rate (1.25 eggs) was estimated from Land and Falconer's (1969) selection experiment for ovulation rate. An estimate of realised genetic correlation between ovulation rate and testis weight ($r_{(A)OT} = \sqrt{CR_O \cdot CR_T / R_O R_T}$) was also made from the observed response in testis weight (100 mg) and the correlated response in ovulation rate (3 eggs) in the present experiment. The correlated response in testis weight (58 mg) and the direct response in ovulation rate (7 eggs) were taken from Land (1973). The genetic correlation between body weight and testis weight was estimated from the observed genetic regression of body weight on testis weight ($r_{(A)WT} = b_{(A)WT} \cdot \sigma_{(A)T}/\sigma_{(A)W}$) and the

value of the genetic standard deviation of body weight (1.72 g) was estimated from Falconer's (1973) selection experiment for body weight.

The partial genetic correlation between ovulation rate and testis weight for constant body weight was calculated from the observed genetic relationships among body weight, ovulation rate and testis weight by applying the formulae for partial correlation and was obtained by:

$$r_{OT.W} = (r_{OT} - r_{OW}.r_{WT}) \; / (\sqrt{(1-r_{OW}^2)(1-r_{WT}^2)}$$

(see, for example, Snedecor and Cochran, 1967).

Accordingly, the partial genetic regression of ovulation rate on testis weight was calculated by using a derived formulae. The formulae for partial regression is derived from the formulae of partial correlation by converting the correlation terms to the relevant regression terms and is given by: $b_{OT.W} = (b_{OT} - b_{OW}\, b_{WT})/(1-r_{WT}^2)$. The values of genetic correlation between ovulation rate and body weight (0.4) and the genetic regression of ovulation rate on body weight (0.4 eggs/g) were taken from Land (1970c).

The estimated values of genetic correlations, partial genetic correlations and partial genetic regressions are tabulated below:

	Between ovulation rate and testis weight:			
	Primiparous		Nulliparous	
	Absolute	Partial	Absolute	Partial
$r_{(A)OT}$	0.51 ± 0.18	0.48	0.25	0.19
Realised r_A	0.50 ± 0.18	0.47		
$b_{(A)OT}$	(0.029)	0.024	(0.014)	0.01
$r_{(A)WT}$	0.22 ± 0.14	Between body weight and testis weight		

Approximate standard errors of the estimates of genetic correlation calculated from the expected variance

$$(V(r_A) = \frac{1-r^2}{4h^2_T h^2_O} [V(h^2_T) + V(h^2_O)])$$

are shown in the table.

The observed magnitude of genetic correlation between primiparous ovulation rate and testis weight was considerable. The magnitude of the genetic correlation between nulliparous ovulation rate and testis weight was rather low, half that for primiparous ovulation rate. The estimates of partial genetic correlation between testis size and ovulation rate and regression of ovulation rate on testis weight indicated that body weight had a little effect on the magnitude of these parameters and made only a small contribution to change in ovulation rate.

The change in body weight was, on average, 1.5 g per 100 mg of change in testis weight. The estimated genetic correlation between body weight and testis weight was found to be low and provided an indication that genetic variation in testis size might be associated with other variables like level and activity of gonadotrophic hormones as postulated above.

Although a correlated response occurred in ovulation rate in selection for testis size, litter size did not change correspondingly. Moreover, litter size behaved differently with respect to divergence between the high and the low selected lines in the two replicates; and on average, there was no evidence of correlated response. Bradford (1969) and Land and Falconer (1969) reported that selection for ovulation rate also did not produce any change in litter size. Selection for body weight at 6 weeks of age (Falconer, 1973) and weight gain from 3 to 6 weeks of age (Bradford, 1971) failed to increase

litter size in the selected high lines but variability in litter size increased due to increase in ovulation rate. Bradford (1972) suggested that increase in ovulation rate might not produce an increase in litter size in mice. Natural ovulation rate, on average, provides the females in this species with as many embryos as they can carry under good health and nutrition.

Consideration of embryonic mortality produced a coherent picture. It appeared that, on average, embryonic mortality was higher in the high selected lines in which the ovulation rate was higher. A positive linear trend of loss of fertilized eggs had been shown to occur in mice by Bowman and Roberts (1958). Selection for litter size (Falconer, 1963) increased ovulation rate in both high and low lines relative to the Control and the high line was found to have the highest proportion of preimplantation losses and the low line to have the highest proportion of postimplantation losses. There seemed to be a positive relationship between ovulation rate and embryonic mortality in this species.

The main conclusion from this experiment concerns the possibility and importance of using testis growth and size as selection criteria for selecting males for the genetic improvement of components of female fertility. It is evident from the present study that selection in males for testis weight produced a change in ovulation rate of females as correlated response. The magnitude of the presence of positive genetic relationship between ovulation rate and testis size was considerable and this relationship was presumed to arise mainly through the physiological pathways controlling the activities of the testis and ovary.

CHAPTER 3

BREED AND SEASONAL VARIATION IN TESTIS ACTIVITY IN SHEEP

I. INTRODUCTION

Breed differences in female reproductive performance in the sheep are well known. There are remarkable differences in age of first oestrus, duration of oestrus, ovulation rate, litter size, frequency of lambing, and period and duration of ovarian activity and breeding season (Land, 1970a, Wheeler and Land, 1973; Land, Russell and Donald, 1974). It is believed that the differences in ovarian activity and reproductive performance between breeds arise partly from differences in the level of gonadotrophin stimulation (Land, 1971; Land, Crighton and Lamming, 1972; Land, Pelletier, Thimonier and Mauleon, 1973).

Since the same gonadotrophic hormones, FSH and LH, control the reproductive activity in the female as in the male (see, for example, Cole, 1969), it is logical that the differences in reproductive activity between breeds should also be expressed in the male and there should be a close relationship among the reproductive activities of males and of females between breeds. Such differences in the male may be observed in testis growth and size, rate of sperm output, level of testosterone production and libido.

Studies on mating behaviour and semen characteristics of Finnish Landrace and Scottish Blackface rams (Land, 1970b) showed that the libido of Finnish Landrace rams, the breed which is known to be highly prolific, was greater than that of Blackface rams. Seasonal changes in libido were marked and they correspond closely to the period and duration of breeding season of the female in each breed. The investigation demonstrates that the differences in level of reproductive activity were not

limited to the female but also exhibited in the male and this activity was positively correlated with that of the females of their breed. Further studies on growth of the testis in ram lambs of Finnish Landrace, Merino and their crossbreeds (Land, 1973) indicated a rapid growth of testis in the Finns and crossbreeds but slow growth of testis in Merinos. The diameter of testis in Finn ram lambs was significantly higher than that in Merinos at any given body weight. Thus testis diameter was greater in the breed in which the ovarian activity, ovulation rate and litter size of females are higher.

Following these indications of genetic relationships between the quantitative expression of reproductive characteristics in the male and the female, an experiment was initiated in which the activity of testis with respect to size and rate of sperm output could be studied in different breeds and crossbreds of sheep which have different levels of female reproductive performance. It was considered that the level of reproductive performance could be estimated in the male as well as in the female. Studies on the characteristics and activity of the testis in different breeds may provide an indirect measure of the level and stimulation of circulating hormones controlling the reproductive activity in the male.

II. MATERIALS AND METHODS

(i) Experimental rams

The experimental work was carried out at A.B.R.O. Dryden Field Laboratory, Roslin, Midlothian. Two pure breeds, namely Finnish Landrace, Tasmanian Merino, and two crossbreeds, Finn-Merino and Finn-Dorset, were included in the experiment. The Finn-Dorset rams were divided into two groups on the basis of the high and low growth rate of their testes from six to fourteen weeks of age. In total 25 rams were taken into the

experimental programme. The number of rams taken in each breed and their ages at the beginning of the experiment are given in Table 3.1.

Table 3.1. Number of rams in each breed and their ages.

Breeds:	Number of rams	Ages: 1 year (No. of rams)	2 years (No. of rams)
Finnish Landrace (F)	5	3	2
Tasmanian Merino (M)	5	3	2
Finn-Merino (FM)	5	2	3
Finn-Dorset high (FDH)	5	5	-
Finn-Dorset low (FDL)	5	5	-

One ram from the Finn-Dorset low testis group died after three weeks of the experiment and no replacement was made for the dead ram. All rams had undergone a libido test prior to the start of the experiment. They were in good health and exhibited an ardent desire for mating. The experimental rams were housed in the same animal house and maintained in individual pens. They were fed with a complete ruminant diet recommended for adult sheep, available as pellets. In order to maintain approximately constant body weight throughout the experimental period, individual rams were fed only three-quarters of their previous daily ad-libitum consumption. Restricted feeding was practised from November to March for individual rams. Restriction was withdrawn and all rams were fed ad-libitum from April to October. No light control was practised, rams were exposed to natural light coming through the window of the house.

(ii) Measuring testis diameter and body weight.

In order to study the characteristics of the testis and its seasonal variation, a year round programme was undertaken from October to September. Body weight in kilograms and testis diameter measurements in centimeters were taken at fortnightly intervals. Testis diameter measurements were taken as follows: At first the left and the right side skin thickness of the scrotum were measured in millimeters using skin thickness callipers, and after that the left and the right testis diameter were measured by slide callipers over the scrotum. The testis diameter was calculated by subtracting the scrotal skin thickness from the observed testis diameter in each side. The average diameter of the left and the right testes was taken as the measurement of testis diameter for an individual ram.

(iii) Techniques for measuring the daily output of spermatozoa.

Lino and Braden (1972) developed a technique for estimating the daily output of spermatozoa in rams by counting the number of spermatozoa voided in the daily urine and suggested that the daily output of spermatozoa in the urine in sexually rested rams provides a reliable estimate of testicular production of spermatozoa. It was decided to follow the same technique for estimating the testicular output of spermatozoa in the present study. An investigation was carried out to develop a collection method, a sampling technique for urine and a method for counting the number of spermatozoa voided in the daily urine prior to the start of the experiment.

A two year old Merino ram and a one year old Finnish Landrace ram were used for this purpose. For collection of urine, each ram was placed in a metal crate, especially designed for sheep, where the animal

could stay quite comfortably about 3 feet above ground level for several days. A special type of cone connected with a rubber tube was made out of 'Hydex' sheet (a kind of waterproof flexible sheet). The cone was strapped under the abdomen covering the prepuce. The flexible cone was used for collecting urine so that the animal could lie down and get up easily during the collection period. The urine, when voided, was received by the cone and ran through the rubber tube into a five litre polystyrene bottle, containing 200 ml of Saponin solution (0.7% Saponin made up in 0.4% aquous formalin, Lino and Braden, 1972). The urine was collected over a period of 12 consecutive days for each of the two rams. The bottle was changed daily at 12.00 hours.

Five independent samples of 20 ml each were taken from each day's collection of urine. The bottle containing the daily collection of urine and Saponin solution was shaken well before taking each sample. The total number of spermatozoa in the daily urine was determined by haemocytometer count. The volume of haemocytometer square chosen was 1/80 mm^3 (1/16 mm^2 area x 1/5 mm depth). The urine sample was diluted with tap water, when the number of spermatozoa was found to be more than 10 per haemocytometer square. The dilution rate was 5, 10 or 20 times. Two independent drops from each urine sample were used on each of the two sides of the haemocytometer. 50 spermatozoa were counted in each side of the haemocytometer and a count was taken of the number of squares required to achieve this number. The total number of spermatozoa voided in the daily urine was determined as follows: The volume of a small square in the haemocytometer is 1/80 mm^3 which is equivalent to 1/80,000 ml. When the number of spermatozoa obtained per small square is Y, the number of sperm in one ml of urine would be

80,000 Y. When the volume of daily urine collected is Z ml, the total number of sperm in the daily urine would be 80,000 YZ. Since a portion of Saponin solution was used in the urine and the urine was diluted with water for counting the spermatozoa, the total number of spermatozoa in the daily urine was determined on the amount of urine plus Saponin solution, and taking into account the dilution rate. The daily output of spermatozoa was estimated from the formula stated below and the calculation was done through a programme for Olivetti desk computer.

$$N = n/m \times (U + S) \times d \times 80,000$$

where N = Number of sperms in the urine

n = Number of sperms counted

m = Number of small squares

U = Amount of Urine in ml.

S = Amount of Saponin solution in ml.

d = Dilution rate

80,000 = A constant for volume of small square expressed in ml.

The data collected on the sperm output in each day's urine with five independent samples and each sample counted in two replicates were analysed statistically within each ram. Analyses of variance were made using a nested classification of sperms counted between days, between samples within days and replicates within samples (Table 3.2). The results indicated that there were no significant differences between samples within days but there were highly significant differences between days within each ram. The average daily yield of spermatozoa was 2109×10^6 in Finn ram (range 51×10^6 to 10513×10^6) and 4327×10^6 in Merino ram (range 73×10^6 to 16781×10^6).

Table 3.2. Analysis of variance of the output of spermatozoa (Number x 10^8) in the daily urine.

Source	d.f.	Finn		Merino	
		MS	F	MS	F
Between days	11	6825	341.250**	22177	299.689**
Between Samples	48	20	1.333	74	1.175
Within Samples	60	15		63	

** = P < 0.01

Tischner (1971) remarked that the rams might have masturbated by rubbing the penis against the rubber funnel fastened under the prepuce (remarks passed on the method of urine collection described by Lino et al., 1967; almost a similar method described above). He reported that after masturbation had been prevented by tying the tunica albugenia of the penis to the skin, a significant reduction of spermatozoa was found in the urine. Following the conclusion, an arrangement was made to observe the behaviour of the rams (the same rams mentioned above) during collection of urine. A transparent polythene paper cone connected to rubber tubing was fastened under the abdomen covering the prepuce. The transparent cone was used so that the action of the penis could be seen from outside and any erection or masturbation could be marked. The rams were constantly observed during twenty-four hours on a single day (August 21st, 1973). A sample of about 10 ml of urine was taken away during each urination and the remainder was allowed to pass through the cone to the bottle containing Saponin solution. A fresh cone was used after every urination and the prepuce washed and dried every time before placing the cone. The sample collected during urination and the whole

urine in the bottle was examined under the microscope.

It was noted that the Merino ram erected its penis three times at night but no sign of masturbation or any secretion of semen followed. The Finn ram showed no erection that night. Examinations on the sample of urine indicated that the spermatozoa were present in the urine of both rams but the concentration of spermatozoa between urinations varied very much. It was assumed that the rams may not masturbate against the cone fastened under the prepuce but the rate at which spermatozoa came out in the normal urine differs very much between urinations and is at irregular intervals.

(iv) Programme for measuring the daily output of spermatozoa

The system for collecting urine and measuring the daily output of spermatozoa, described above, worked reasonably well with little or no stress to the animal and it was decided to collect urine and measure daily output of spermatozoa by this method for the present experiment.

The programme for measuring the daily output of spermatozoa was designed so that all individuals could be measured during two consecutive months, covering November-December, February-March, May-June and August-September; the months represented the peak, late, non- and early-breeding season, respectively. The urine of each ram was collected for 10 consecutive days in each term. 5 rams were taken at a time for collection of urine with one ram from each group. The complete cycle for 25 rams covered approximately two months.

The urine collected for two consecutive days (e.g. days 1 + 2, 3 + 4) for each ram was combined together and mixed well by stirring in a container. A sample of 20 ml urine was taken from the mixed urine. In this way five samples were taken from ten days collection of urine

for each ram. The number of spermatozoa present in the urine was determined by haemocytometer count using the same type of haemocytometer and following the same procedures as described before. Around 100 spermatozoa were counted in each side of the haemocytometer using two independent drops from a sample. The number of spermatozoa present in the total volume of urine was calculated according to the formulae given above.

III. RESULTS

(i) Testis diameter and body weight

Average (mean of year round measurements) body weight, testis diameter and daily sperm output of individual rams are given in Table 3.3a.

The mean testis diameters and the mean body weights along with the means adjusted for age difference of rams in each breed group are given in Table 3.3b. The mean testis diameters corrected for body weight ($CT = UT - b(W - \bar{W})$ over the adjusted means are also given in the same table. There were significant differences in testis diameter and body weight between breeds (Table 3.3c). The mean testis diameter in younger rams was lower and the mean difference between the two age groups of rams was found to be statistically significant ($P < 0.01$) but no significant effect of age was observed in body weight. Tests for least significant difference among the breed averages are given in Table 3.4. The mean testis diameter of Merino rams was significantly lower than that of the other breeds. There were no significant differences in testis diameter between Finns and Finn-Merinos and between Finn-Dorset high and low groups, but testis diameter in Finn-Dorsets were significantly higher than in Finn-Merinos. The mean body weight of Merino

rams was also significantly lower than that of others. Finns and Finn-Merinos had approximately the same body weights and so had the Finn-Dorset high and low groups. But the mean body weights in Finn-Dorset high and low groups were significantly higher than that in Finns and Finn-Merinos.

Table 3.3(a). Body weight, testis diameter and daily sperm output of individual rams.

Breeds	Rams' Identity	Body Weight (Kg)	Testis Diameter(cm)	Daily Sperm output (N x 10^6)
Finnish Landrace	1U 009	54.59	5.83	2615
	1U 069	70.61	5.58	5729
	2U 103	66.61	5.29	3027
	2U 074	61.65	5.59	2327
	2U 081	66.54	5.23	2652
Finn-Merino	1U 075	64.93	5.65	4451
	1U 076	73.30	5.65	2585
	1U 088	69.96	5.82	3000
	2U 051	53.11	5.25	3184
	2U 114	64.87	5.50	4636
Merino	1U 052	52.69	5.04	1557
	1U 136	57.65	4.93	3402
	2U 066	41.20	4.20	519
	2U 152	35.24	4.01	900
	D 469	49.46	4.57	1699
Finn-Dorset High	22790	77.98	5.82	514
	22799	70.63	5.60	3705
	22809	85.33	5.69	3690
	22836	74.44	5.37	2957
	22944	80.74	5.76	2586
Finn-Dorset Low	22795	67.44	5.52	3636
	22817	82.44	5.80	1724
	22833	74.30	5.30	4333
	22866	88.22	5.69	5080

The rams marked 1U in the table were older.

Table 3.3(b). Mean testis diameter (Cm.) and body weight (Kg.) (Mean of individual rams on average testis diameters of year round measurements; S.E. over number of rams).

Breed	Testis diameter		Body Weight		Mean testis diameter (corrected for body weight)
	Mean ± S.E.	Least Square Mean (adjusted for age)	Mean ± S.E.	Least Square Mean (adjusted for age)	
Finn (F)	5.51 ± 0.11	5.55 ± 0.09	64.00 ± 2.75	64.71 ± 3.19	5.66
Finn-Merino(FM)	5.57 ± 0.10	5.53 ± 0.09	65.23 ± 3.42	64.52 ± 3.19	5.65
Merino (M)	4.55 ± 0.20	4.60 ± 0.09	47.25 ± 4.02	47.96 ± 3.19	5.29
Finn-Dorset high (FDH)	5.65 ± 0.08	5.88 ± 0.10	77.82 ± 2.53	81.37 ± 3.68	5.42
Finn-Dorset low (FDL)	5.58 ± 0.11	5.81 ± 0.11	78.10 ± 4.56	81.65 ± 4.01	5.34

(c) Least-square analysis of variance

Source	d.f.	Testis diameter		Body weight	
		MS	F	MS	F
Breed	4	1.2009	30.326**	794.9236	15.803**
Age	1	0.7747	19.563**	181.0786	3.600
Remainder (Interaction)	18	0.0396		50.3021	

** = P < 0.01

Table 3.4. Tests of least significant difference among the adjusted means.

(a) Testis diameter adjusted for age.

Breed groups	FDH	FDL	F	FM	M
Means (Cm.)	5.88	5.81	5.55	5.53	4.60

(b) Testis diameter corrected for body weight.

Breed groups	F	FM	FDH	FDL	M
Means (Cm.)	5.66	5.65	5.42	5.34	5.29

(c) Body weight adjusted for age.

Breed groups	FDL	FDH	F	FM	M
Means (Kg.)	81.65	81.37	64.71	64.52	47.96

The means that are not underlined by the same line are significantly different from each other ($P < 0.05$).

(ii) Seasonal variation in testis diameter.

The mean testis diameters for each month in each breed along with their body weights are shown in Fig. 3.1a and b. It is evident from the figure that testis diameter showed a distinct curve in each breed which was at its lowest in April, May, June and July. The measurements on testis diameter for each ram were fitted by a sine curve, to test whether there was any evidence of cyclical variation during the year. The measurements were also used to obtain the day from January 1st at which the testis diameter was minimum. The results of the tests are

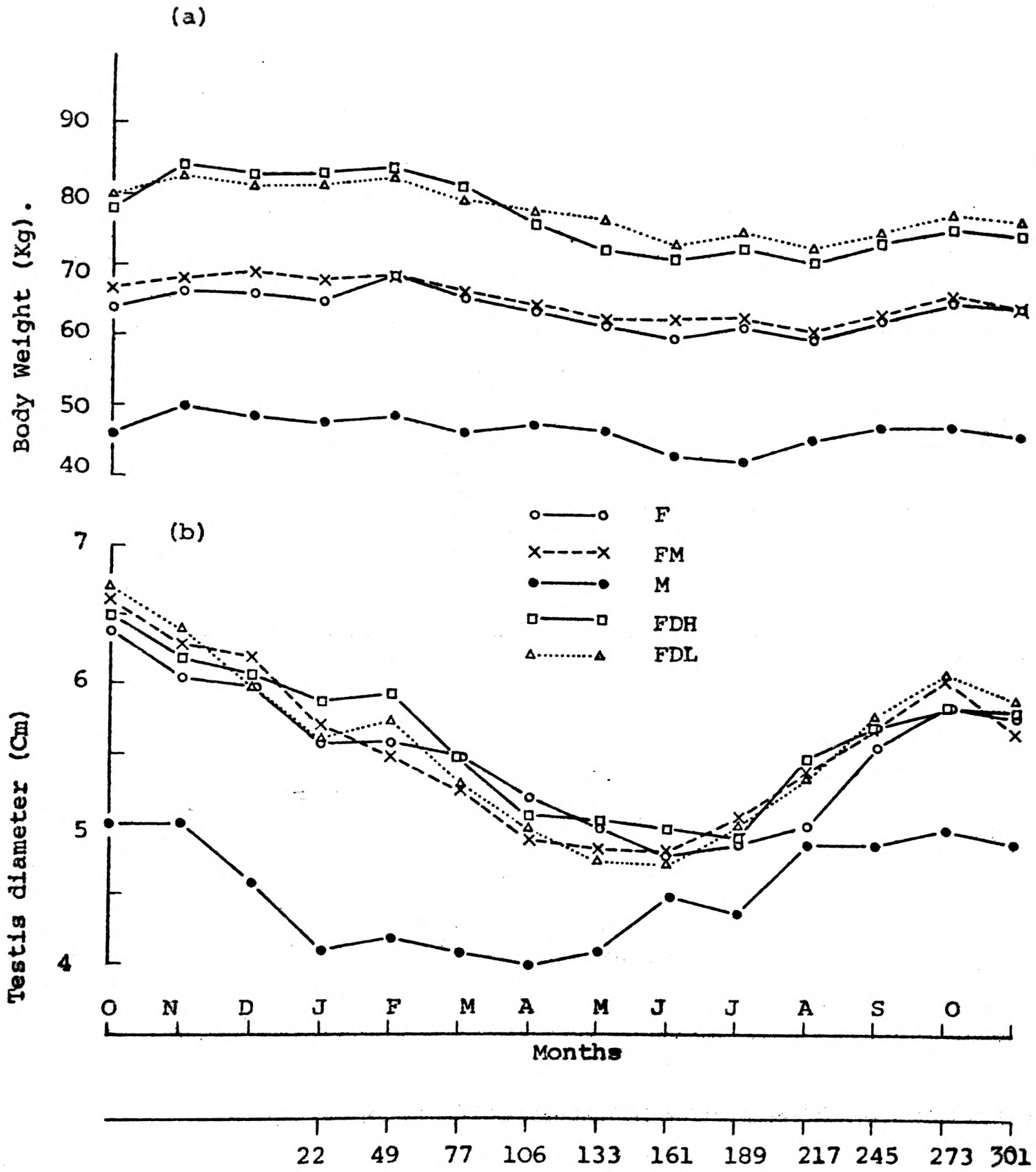


Fig. 3.1. (a) Average body weights in Finnish Landrace (F), Merino (M), Finn-Merino (FM), Finn-Dorset high (FDH) and Finn-Dorset low (FDL).

(b) Average testis diameters in different breeds.

Table 3.5. Seasonal variation in testis diameter and body weight.

Breeds & Rams		Testis diameter		Body weight	
		Test for cycle (level of significance).	Day from 1st Jan. when min.	Test for cycle (level of significance).	Day from 1st Jan. when min.
Finns	009	P < 0.1%	138	P < 0.1%	195
	069	P < 0.1%	163	P < 0.1%	206
	103	P < 0.1%	153	Not sig.	242
	074	P < 0.1%	149	P < 1.0%	216
	081	P < 0.1%	151	Not sig.	153
	Breed average		151		202
Finn-Merinos	075	P < 0.1%	134	P < 0.1%	171
	076	P < 0.1%	146	P < 0.1%	215
	088	P < 0.1%	141	P < 5.0%	192
	051	P < 0.1%	138	Not sig.	202
	114	P < 0.1%	138	P < 0.1%	184
	Breed average		140		193
Merinos	052	P < 0.1%	104	P < 0.1%	200
	469	P < 0.1%	79	P < 5.0%	111
	136	P < 0.1%	145	P < 0.1%	212
	066	P < 0.1%	99	Not sig.	365
	152	P < 1.0%	83	Not sig.	184
	Breed average		102		254
Finn-Dorset High	790	P < 0.1%	148	P < 0.1%	198
	799	P < 0.1%	160	P < 0.1%	210
	809	P < 0.1%	138	P < 0.1%	216
	836	P < 0.1%	166	P < 0.1%	191
	944	P < 0.1%	153	P < 0.1%	198
	Breed Average		153		203
Finn-Dorset Low	795	P < 0.1%	130	P < 0.1%	162
	817	P < 0.1%	151	P < 0.1%	219
	833	P < 0.1%	142	P < 0.1%	219
	866	P < 0.1%	132	P < 0.1%	218
	Breed Average		139		209

given in Table 3.5. It is recognised that every individual ram had shown a significant change in testis diameter and there was a clear evi- of seasonal variation in testis diameter in the sheep. On average, the testis diameter was minimum at day 102 in Merino, at day 140 in Finn-Merino, and at day 151 in Finn rams. Finn-Dorset high and low groups showed a difference of 14 days in their minimum testis diameters which was at day 153 in high and day 139 in low groups (Table 3.5). Testis diameter in Merino rams had gone down and started to rise up 50 days earlier than that in Finn rams. Finn-Merino was within the range of parental breeds but much closer to Finns. On average, Finn-Dorset rams showed an earlier cycle than that in Finns but later than Finn-Merino. The cycle in Merino rams was significantly different from other breeds but there was no significant difference from the rest of the breeds (Table 3.6b).

There appeared to be a significant reduction of bodyweight in the majority of the rams (Table 3.5), but the cycle was less clear. The cycles for body weight lagged behind the testis diameter by about 50 days. Moreover, when there was no significant change in body weights of some rams, testis diameter of the same rams significantly followed the cycle. It appeared that there was no relationship between the cycles for testis size and body weight.

Table 3.6(a). Analyses of variance of the day at which testis diameters and body weights were minimum.

		Testis diameter		Body weight	
Source	d.f.	MS	F	MS	F
Between breeds	4	2099	10.24**	295	0.13
Within breeds	19	205		2248	

** = $P < 0.01$

Table 3.6(b). Tests for least significant difference among the mean days for testis diameter.

Breed groups	FDH	F	FM	FDL	M
Mean (days)	153	151	140	139	102

The mean that is not underlined by the same line is significantly different from others ($P < 0.01$).

(iii) Breed and seasonal variation in sperm output.

The mean daily sperm output in four seasons covering November-December, February-March, May-June and August-September in each breed are shown in Fig. 3.2 and Table 3.7. Least-square analyses of variance of the average daily sperm output of individual rams showed that there was no significant difference between the two age groups of rams (Table 3.8a). Further analyses of variance (Table 3.8b) including seasons indicated that a highly significant difference existed between the seasons. There was no significant difference between breeds but the

Table 3.8 (a). Analysis of variance of daily output of spermatozoa (Number x 10^8)

Breed and age effects

Source	d.f.	MS	F
Breed	4	365.0432	2.445
Age	1	226.354	1.516
Remainder (Interaction)	18	149.312	

(b) Breed and Season effects

Breed	4	1389.264	2.265
Season	3	2776.624	14.111**
Breed X Season	12	406.294	2.065*
Rams within	19	613.348	3.117**
Error	57	196.773	

* = $P < 0.05$ ** = $P < 0.01$

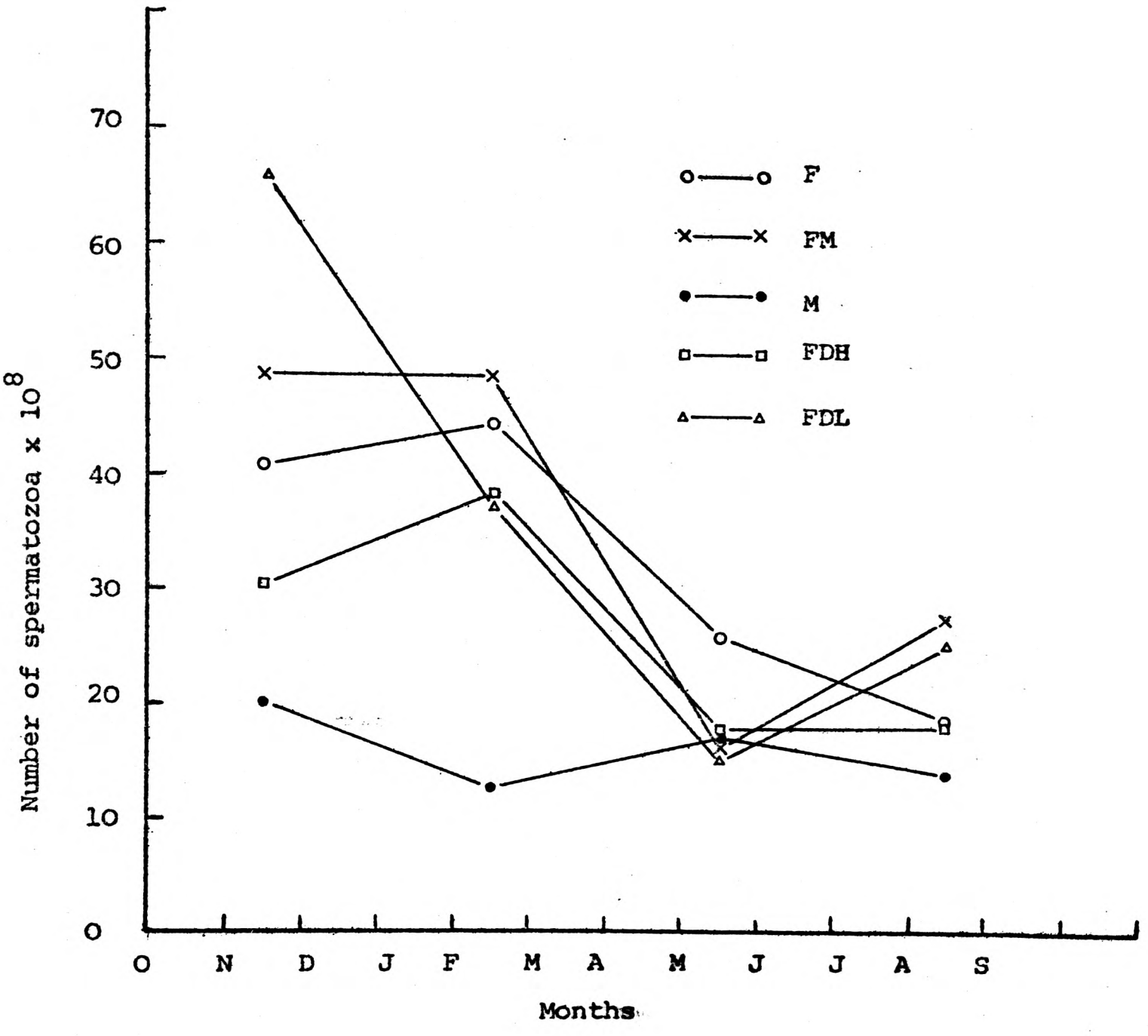


Fig. 3.2. Average daily output of spermatozoa in Finnish Landrace (F), Merino (M), Finn-Merino (FM), Finn-Dorset high (FDH), and Finn-Dorset low (FDL).

Table 3.7. Average daily sperm output (Number x 10^6) (S.E. over number of rams).

Breeds	No. of rams	Seasons				Breed Average
		Nov-Dec	Feb-Mar	May-June	Aug-Sept	
Finn	5	4130 ± 705	4447 ± 1310	2597 ± 414	1906 ± 650	3270 ± 625
Finn-Merino	5	4898 ± 589	4909 ± 1023	1658 ± 306	2821 ± 365	3571 ± 410
Merino	5	2028 ± 712	1338 ± 651	1666 ± 658	1425 ± 513	1615 ± 496
Finn-Dorset high	5	3070 ± 928	3940 ± 1120	1865 ± 605	1888 ± 458	2688 ± 585
Finn-Dorset low	4	6611 ± 1283	3686 ± 1302	1584 ± 487	2885 ± 951	3693 ± 720

Table 3.9. Phenotypic correlations among body weight, testis diameter and sperm output.

Breed groups	Correlations between		
	Body wt. and testis diam.	Body wt. and sperm output	Testis diam. & sperm output
Finn	-0.289	0.492	-0.710
Finn-Merino	0.358	-0.201	0.556
Merino	0.902*	0.723	0.435
Finn-Dorset high	0.492	-0.045	-0.508
Finn-Dorset low	0.633	0.093	-0.485
Pooled (Within breed)	0.500	0.270	-0.137

* $P < 0.05$

interaction between breed and season was found to be significant. The sperm output of individual rams differed significantly within breeds.

(iv) Interrelationships between body weight, testis diameter and sperm output.

Phenotypic correlations among body weight, testis diameter and daily sperm output were estimated within each breed from the measurements of individual rams adjusted for age and are given in Table 3.9. Positive correlations between body weight and testis diameter were obtained in Merino, Finn-Merino and Finn-Dorset but relationship in Finn was found to be negative. The relationships between testis diameter and sperm output were found to be positive in Merino and Finn-Merino, but negative in other breeds. A higher positive correlation between body weight and sperm output was found in Merino. A test for homogenosity of different estimates of correlation coefficients showed that there were no significant differences among the estimates in different breeds. So, the estimates in different breeds were pooled together and the pooled estimates of phenotypic correlations within breeds were 0.50, 0.27 and -0.14 between body weight and testis diameter, between body weight and daily sperm output, and between testis diameter and daily sperm output, respectively.

IV. DISCUSSION AND CONCLUSIONS

The mean testis diameters in Merino rams was found to be significantly lower ($P < 0.01$) than that in Finns, Finn-Merinos and Finn-Dorsets. A further analysis showed that testis diameter corrected for body weight was also significantly lower ($P < 0.01$) in Merino than in Finn and Finn-

Merino but not than in Finn-Dorset. The conclusion that at any given body weight testis size in Merino rams is significantly smaller than that in Finn and Finn-Merino agrees with the observations of Land (1973) in which the testis growth and size were studied in young ram lambs but contradicts with the later observation that testis size appeared to be the same at two years of age, i.e. Merinos growing in their second year.

The characteristic seasonal change in testis diameter found in each breed, crossbred or group is of interest. In general, maximum testis diameter was observed in the months of the regular breeding season and minimum in the months of the non-breeding season. A systematic seasonal change in testis weight was reported by Carson and Amann (1972) in the rabbit in which the mean testis weights were significantly lower for rabbits killed in July, August and September than for those killed in the other months of the year. The interesting feature is the pattern of seasonal change in testis diameter of each breed. Merinos showed a significantly earlier fall and rise than the others, the minimum size being in April. Finns showed the latest fall and rise with the minimum in June and the crossbred Finn-Merinos in May. The characteristics of seasonal change in testis diameter in each breed have a positive relationship with the seasonal change in ovarian activity of the ewes in each breed. Wheeler and Land (1973) reported that oestrus and ovulation in Merino start in September and continue up to February with an average ovulation rate of 1.07 eggs, whereas in Finn these start in October and continue up to April with an average ovulation rate of 2.94 eggs. The ovulation rate and litter size in Finn-Merino were shown to be intermediate (Land, Russell and Donald, 1974) between the two parental breeds but the pattern of incidence of oestrus and lambing were much closer to

Finns. On average, Finn-Dorset rams showed a more or less similar pattern of seasonal change in testis size to the Finn-Merinos, but the high and low groups of Finn-Dorsets, grouped on the basis of their rate of testis growth from six to fourteen weeks of age, had different seasonal cycles. The low group showed an early change in testis size compared to the high group.

However, it is reasonable to conclude from the observed results that the characteristics of declining and rising in testis size at the particular period of the year are specifically different in different breeds or crossbreeds and positively related with the breeding season and activity of the particular breed. It indicates that the seasonal variation in reproductive activity is not confined to females, but also exhibited in males and hence it may be possible to estimate the level of reproductive activity either in the male from the measurements of testis properties, or in the female from the measurements of the ovarian activity. These observations, together with the observations on libido and semen characteristics (Land, 1970b) and an ovarian activity (Wheeler and Land, 1973) enable a definition of the relationships between testis activity in males and ovarian activity in females in different breeds. Similarity in expression of some aspects of reproductive activity in the male and the female within each breed and the differences of reproductive activity in the female as well as in the male between breeds justify to remark that these relationships might arise through the differences of the level of circulating gonadotrophins and sensitivity of testis and ovary to these hormones.

The mean daily sperm output did not differ significantly between breeds but the sperm output of individual rams differed significantly

within breeds. The sperm output was found to be susceptible to seasonal changes and differed significantly between breeding and non-breeding seasons. The results obtained were in agreement with many other observations that there is seasonal variation in semen characteristics, but very little evidence for differences between breeds, and variation between individuals seems to be greater than between breeds (Smyth and Gordon, 1967; Land, 1970b).

The interrelationships among body weight, testis diameter and daily sperm output within each breed showed different pictures in different breeds. The observed relationships were not very reliable because the number of individuals within each breed was very small. Positive correlations between body weight and testis diameter (0.90), between body weight and sperm output (0.72) and between testis diameter and sperm output (0.44) were observed only in the Merino.

The correlation between testis diameter and testis weight in the sheep was reported to be 0.94 (Land and Carr, 1975), indicating that the testis diameter is a good estimate of its weight and it is also evident that there is a high positive correlation between testis weight and sperm output in rams (Lino, 1972), in rabbits (Carson and Amann, 1972) and in bulls (Foote et al., 1970). Lino (1972) reported a linear correlation of 0.83 between testis weight and daily output of spermatozoa in Merino rams, from estimating the number of spermatozoa voided in the urine. The same techniques were followed in the present experiment for measuring the daily output of spermatozoa. The magnitude of correlation between testis diameter and daily output of spermatozoa observed in Merino and Finn-Merino rams was in agreement with the observation of Lino (1972), but it did not agree for the other breeds and crossbreds

studied in the present experiment. It implies that the rate at which spermatozoa voided in the urine may be different in different breeds.

In conclusion, the present experiment in the sheep shows that there is seasonal variation in testis activity with respect to size and output of spermatozoa, and the pattern of seasonal change is different in different breeds. The expression of testis activity is similar to that of ovarian activity in each breed, and it appears that there is a positive relationship between the male and the female reproductive activities in different breeds. There should be significant differences in daily output of spermatozoa between breeds. Large variation in sperm output of individual rams within breeds and difficulties in accurate measurements of daily output of spermatozoa limit the possibility of obtaining such differences between breeds.

CHAPTER 4

GENERAL DISCUSSION

The results of the experiment in the mouse indicated that about half of the total variation in testis size was genetic in origin and the selection for testis size not only resulted in a significant direct response but also caused a considerable correlated response in ovulation rate of females. The magnitude of the genetic correlation between ovulation rate and testis size was noted to be 0.5 and this relationship was not solely mediated through body weight. Observations of this sort demonstrate that ovulation rate of females could be changed indirectly by selection for testis size in males. Thus testis size could be regarded as a selection criterion for selecting males to produce the same effect as direct selection for ovarian activity in females. Moreover, the results of the experiment in the sheep showed a similar pattern of seasonal change in testis size in each breed to that in ovarian activity of females. It implies that the level of fertility could be measured in the male by looking at testis activity in terms of size, rate of sperm output or libido as well as in the female by looking at ovarian activity in terms of frequency of oestrus and rate of ovulation and lambing. The observations of the present experiments in the mouse and the sheep provide a clue that selection for genetic improvement of female reproductive performance is possible in males or simultaneously in males and females. An important consideration is that it overcomes the problem of sex limitation in selection for female reproductive performance and selection pressure can be applied in both males and females.

Let us consider how the application of this new selection criterion can help in the genetic improvement in animal production, viz. lamb

production, where it seems that an increase in ovulation rate could increase the number of lambs born per parturition. Suppose that the heritability of testis size ($h^2 = 0.5$) and ovulation rate ($h^2 = 0.25$), and the genetic correlation between testis size and ovulation rate ($r_A = 0.5$) observed in the mouse are the same in the sheep. It could be assumed that indirect selection for ovulation rate would be more efficient than direct selection when the intensity of selection is considered to be twice than that in females. The ratio of genetic gain in indirect to gain in direct selection is:

$$\frac{CR_O}{R_O} = \frac{i_T h_T r_A \sigma_{(A)O}}{i_O h_O \sigma_{(A)O}} = \frac{i_T}{i_O} r_A \frac{h_T}{h_O}$$

when the intensity of selection is two times greater in the male, this ratio exceeds one.

$$CR_O/R_O = 2 r_A h_T / h_O = 2 \times 0.5 \times 0.7/0.5 = 1.4 .$$

Although the genetic correlation appears to be moderate, the heritability of the second trait is two times greater than that in the desired one. In practice, much higher selection intensity can be achieved in males. However, in a situation where selection can be practised indirectly in males and directly in females for the same desired character, the effective method may be a combination of the two where the rate of genetic gain would be:

$$\text{Rate of genetic gain} = \frac{\text{Correlated response to selection in males}}{\text{Generation interval in males}} + \frac{\text{Direct response to selection in females}}{\text{Generation interval in females.}}$$

In such a situation, the rate of genetic gain can also be increased by

quick generation turnover in males along with much intense selection. In the sheep, the low reproductive rate imposes a limit such that the average age of mothers at which progeny are born can not be reduced by much below 4 years, whereas in a programme in which the ram lambs are used, the age of rams can be reduced to one year. Let us put some relevant values in the above formulae assuming that in a population of sheep 5% of the males (i = 2.063) and 50% of the females (i = 0.798) can be selected. The rate of genetic gain in such cases would be:

$$\Delta G = \frac{i_T h_T r_{(A)OT} \sigma_{(A)O} + i_O h_O \sigma_{(A)O}}{Lm + Lf}$$

$$= \frac{(2.063 \times 0.7 \times 0.5 + 0.798 \times 0.5)\sigma_{(A)O}}{1 + 4}$$

$$= 0.224\ \sigma_{(A)O} \quad \text{(Combined indirect and direct selection).}$$

Indirect selection in males,

$$\Delta G = \frac{2.063 \times 0.7 \times 0.5\ \sigma_{(A)O}}{1 + 4} = 0.144\ \sigma_{(A)O}$$

Direct selection in females,

$$\Delta G = \frac{0.798 \times 0.5\ \sigma_{(A)O}}{1 + 4} = 0.08\ \sigma_{(A)O}$$

when $r_{(A)OT} = 0.25$, $\Delta G = 0.132\ \sigma_{(A)O}$ for combined indirect and direct and $\Delta G = 0.072\ \sigma_{(A)O}$ for indirect selection in males.

It appears from the estimates of the rate of response that a combination of indirect and direct selection for ovulation rate (ovulation rate has a high positive relationship with litter size in the sheep and can possibly be measured by Laparoscopy at sexual maturity and subsequently by observing the lambing rates later) in males and females, respectively,

may expedite an increase in the rate of genetic gain by 180% over direct selection in females alone. Indirect selection in males alone may provide 80% improvement in the rate of response. Indirect selection may be almost as efficient as the direct selection even when the genetic correlation between testis size and ovulation rate is considered to be 0.25, in such practice.

When rams are judged on the lambing performance of their dams, e.g. choosing rams from triplets or twins and on the reproductive performance of their sisters, the characteristics of testis activity would provide an additional source of information to evaluate their genetic merit. In such practice, the rate of testis growth and its size in ram lambs can be considered in a sequential selection programme. Moreover, testis activity can be taken into consideration for the construction of genetic selection index in combination with the level and activity of hormones in reproduction. The development of such a physiological quantitative criterion may provide a great advantage in future selection programmes for fertility.

SUMMARY

Relationships between reproductive activities of the male and of the female were investigated in the mouse and the sheep. In the mouse, selection for testis weight was practised and correlated responses to selection were studied in components of female fertility. In the sheep, the characteristics of testis size and rate of sperm output in adult males were examined for a yearly period in five different breeds and crosses.

A divergent selection for testis weight in the mouse was carried out in two replicates and, on average, 7 males were selected on the basis of their individual testis weights out of 30 males measured in each selection line. Five generations of selection produced a divergent response of 100 mg and yielded a realised heritability of 0.50. Correlated responses occurred in ovulation rate in the female and body weight in both sexes. The genetic regression of ovulation rate on testis weight was 0.030 eggs per mg. in primiparous females and 0.015 eggs per mg. in nulliparous females. Combining data from other experiments, the genetic correlation was estimated to be 0.50 in primiparous and 0.25 in nulliparous females. The genetic regression of body weight on testis weight was 0.015 g per mg. and the genetic correlation was 0.20. There were no correlated changes in litter size.

In the sheep, testis diameter and daily output of spermatozoa were measured in Finnish Landrace, Tasmanian Merino, Finn-Merino and two groups of Finn-Dorset rams, grouped on the basis of their rate of testis growth from 6 to 14 weeks of age. Five rams from each breed group were included in the study. The daily output of spermatozoa was estimated by counting the number of spermatozoa voided in the urine. Breed

differences in testis diameter were significant and testis diameter was lowest in Merino rams. There were seasonal changes in testis diameter with the minimum in the non-breeding season. The breed groups differed in time of their minimum points. The pattern of seasonal change in testis size correspond with the ovarian activity and breeding season of females of the same breed or cross group. The daily output of spermatozoa differed significantly between seasons but there were no significant differences between breeds. Individual rams differed significantly in their daily output of spermatozoa.

It appeared from the present study in the mouse and the sheep that there were positive relationships between the activities of testis and ovary and these relationships were presumably arisen through the stimulation of gonadotrophic hormones which control testis and ovarian activities. Observations in the present study demonstrated that some aspects of fertility could be measured both in the male and the female. The possibility of genetic improvement in female reproductive performance by selection of males on the basis of testis activity was examined.

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